

Intro

Quantitation of double-stranded DNA using Quant-iT PicoGreen Reagent

Invitrogen (Molecular Probes)

MATERIALS

- o Quant-iT PicoGreen dsDNA Assay Kit, including lambda DNA standard (Invitrogen cat. #P7589 or P11496)
- o Black 96-well plate (Greiner Bio-One, cat. # 655096)
- o Brown or amber (light-blocking) microcentrifuge tubes

METHODS*Set up the protocol:*

- o Select Wells to Read and Assay Plate Type by clicking on "Settings" and locating the options on the left side of the screen.
- o Click the Template button to open a window where you can assign wells of the microplate to pre-set template groups using the drop-down menu to select the appropriate template group. There are preconfigured template groups in the PicoGreen Fluorescence protocol including Standards, Unknowns, and Unknowns_NoDiln (for undiluted samples). Assigning wells to pre-set template groups populates group tables in the protocol with the corresponding data acquired when the microplate is read.

Prepare the assay

The method for this assay follows the instructions in the product information sheet for Quant-iT PicoGreen dsDNA Reagent and Kits from Molecular Probes, except that the assay volume is proportionately reduced from 2.0 mL to 200 μ L to fit a 96-well microplate format.

- o Prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) by diluting the concentrated buffer from the kit 20-fold with distilled DNase-free water, as required by Molecular Probes.
- o Prepare an aqueous working solution of Quant-iT PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE buffer (prepared above). Preparation of the solution in a plastic container, rather than glass, is recommended, as the reagent may adsorb to glass surfaces. Protect the solution from light by using amber or brown tubes, or by covering with foil. This solution should be used within a few hours of its preparation.
- o DNA standard curve: Prepare a 2 μ g/mL stock solution of dsDNA in TE. The lambda DNA standard provided with the kit can be diluted 50-fold in TE to make the 2 μ g/mL solution. Note: in some cases it may be preferable to make the standard curve using DNA similar to the type being assayed.
- o A high-range standard curve may be prepared from 1 ng/mL to 1 μ g/mL, or a low-range standard curve may be prepared from 25 μ g/mL to 25 ng/mL. For the high-range curve, follow the dilution scheme shown in the PicoGreen product insert; for the low-range curve, dilute the 2 μ g/mL solution 40-fold to yield a 50 ng/mL solution, and refer to the alternative dilution scheme in the product insert.
- o Pipet standards into a solid black 96-well microplate at 100 μ L per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).
- o Add 100 μ L of the aqueous working solution of Quant-iT PicoGreen reagent to each well. Mix well by trituration or plate shaker and incubate for 2 to 5 minutes at room temperature, protected from light.

Read the microplate

- " Make sure the purple plate adapter is in the microplate reader drawer. Place the microplate in the drawer.
- " Click the Read button in the SoftMax Pro software. The instrument will read the plate and the relative fluorescence units will be displayed in the Plate section of the protocol.

Analyze the data

- o After the microplate has been read, the relative fluorescence units (RFUs) will be displayed in the Plate section. The data will be analyzed in the Group Tables that were created when the template was set up.
- o Standards assigned in the Template (and thus displayed in the Standards group table) will be automatically plotted in the Standard Curve section of the protocol. A linear curve fit is applied by default, but a log-log fit may be used when plotting a standard curve over a wide dynamic range. Curve fits are chosen from the drop-down Curve Fit menu in the graph section's tool bar.

READER SUITABILITY:

All SpectraMax readers with fluorescence capability.

PROTOCOL REVISION HISTORY:

- v 1.1: Imported from SMP 5.4.2 April 2011 (CLO & ELM)
- v 1.2: Emission wavelength changed from 540 nm to 525 nm. (CLO)

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.3e7	3.9e7	3.8e7	2.7e6	2.7e6	3.0e6	5.5e5	5.7e5	5.3e5	6.5e4	6.6e4	6.5e4
B	1.6e7	1.7e7	1.8e7	1.4e6	1.7e6	1.8e6	1.8e6	1.9e6	1.6e6	6.8e4	6.7e4	6.4e4
C	8.6e6	9.0e6	8.9e6	2.8e6	3.0e6	3.0e6	3.3e6	3.3e6	3.0e6	6.7e4	6.7e4	1.2e5
D	4.4e6	4.4e6	4.4e6	2.3e6	2.8e6	2.8e6	2.8e6	2.9e6	2.6e6	6.9e4	6.7e4	6.7e4
E	2.0e6	2.1e6	2.2e6	8.5e5	1.0e6	1.1e6	1.1e6	1.1e6	1.1e6	6.9e4	6.7e4	6.7e4
F	9.9e5	1.1e6	1.1e6	2.0e6	2.5e6	2.4e6	2.7e6	2.7e6	2.4e6	6.6e4	6.6e4	6.9e4
G	6.4e4	6.4e4	6.7e4	5.1e5	6.5e5	6.6e5	5.9e5	6.1e5	5.6e5	6.8e4	6.6e4	6.5e4
H	6.3e4	6.5e4	6.6e4	7.7e5	7.3e5	7.7e5	6.8e5	7.0e5	6.4e5	6.5e4	6.4e4	6.4e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint
 Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:20 PM
 3/17/2014

Mean Temperature : 26.5 °C

Plate02

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.6e6	6.3e6	7.3e6	1.2e7	3.0e6	1.3e6	3.4e6	4.1e6	1.3e6	1.8e6	7.6e5	7.2e4
B	1.6e6	2.9e6	4.6e6	3.9e6	1.9e6	2.0e6	1.5e6	1.9e6	2.9e6	5.0e6	1.5e6	7.2e4
C	6.5e6	1.0e7	2.3e6	9.5e6	7.9e4	1.6e6	2.2e6	2.7e6	6.6e5	2.0e6	2.3e6	7.2e4
D	1.6e6	5.7e6	2.8e6	2.1e6	9.7e5	1.5e6	7.7e4	9.5e5	4.1e6	2.4e6	7.0e5	7.2e4
E	1.6e6	1.4e6	2.5e6	1.4e6	1.6e6	2.5e6	1.8e6	4.6e6	4.4e6	1.2e6	6.5e6	7.2e4
F	7.3e6	6.9e6	3.4e6	2.1e6	1.2e6	5.9e5	4.4e5	6.4e5	4.7e6	2.3e6	1.4e6	7.8e4
G	9.2e6	1.8e6	1.8e6	7.9e6	7.3e5	2.7e6	1.6e6	8.7e5	3.9e5	2.1e6	4.6e6	1.9e5
H	6.8e6	6.5e5	8.6e6	1.5e6	7.5e4	1.1e6	4.3e6	4.3e5	1.7e6	6.8e5	1.3e6	6.9e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint
 Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:24 PM
 3/17/2014

Mean Temperature : 26.5 °C

Plate03

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.1e6	6.0e6	6.3e6	9.4e6	2.5e6	1.2e6	3.0e6	3.5e6	1.2e6	1.5e6	6.8e5	6.8e4
B	1.4e6	2.5e6	4.0e6	3.5e6	1.7e6	1.7e6	1.4e6	1.7e6	2.4e6	4.3e6	1.3e6	6.6e4
C	5.9e6	8.7e6	1.9e6	8.1e6	7.7e4	1.4e6	1.9e6	2.3e6	5.8e5	1.7e6	1.9e6	6.7e4
D	1.4e6	5.0e6	2.4e6	1.9e6	8.0e5	1.3e6	7.5e4	8.2e5	3.5e6	2.1e6	6.2e5	6.7e4
E	1.4e6	1.2e6	2.2e6	1.2e6	1.4e6	2.3e6	1.6e6	4.0e6	3.8e6	1.0e6	5.5e6	6.7e4
F	6.0e6	5.8e6	3.1e6	1.8e6	1.1e6	5.9e5	3.8e5	5.8e5	4.0e6	1.9e6	1.2e6	6.8e4
G	7.3e6	1.4e6	1.6e6	6.9e6	7.1e5	2.6e6	1.5e6	8.0e5	3.5e5	1.8e6	3.7e6	6.6e4
H	5.7e6	5.3e5	6.9e6	1.5e6	7.4e4	1.1e6	3.9e6	4.1e5	1.5e6	5.7e5	1.0e6	6.5e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

- Fluorescence
- Lm1 485, 535
- Slide(s) Ex1, Em1
- More Settings
- Shake Off
- ReadOrder Row
- Show Optimizer On
- PMT and Optics
- Integration Time 400 ms
- Read from Top
- Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:27 PM
 3/17/2014

Mean Temperature : 27 °C

Plate04

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.8e6	6.6e6	7.4e6	1.2e7	2.8e6	1.3e6	3.2e6	3.8e6	1.3e6	1.8e6	7.1e5	3.2e7
B	1.7e6	3.0e6	4.5e6	3.9e6	1.9e6	1.8e6	1.4e6	1.7e6	2.8e6	4.7e6	1.4e6	1.7e7
C	6.9e6	1.1e7	2.3e6	9.4e6	7.9e4	1.5e6	2.1e6	2.6e6	6.0e5	1.9e6	2.1e6	1.1e7
D	1.7e6	5.7e6	2.5e6	2.0e6	9.0e5	1.4e6	7.7e4	9.1e5	4.0e6	2.3e6	6.5e5	4.6e6
E	1.5e6	1.4e6	2.4e6	1.3e6	1.5e6	2.4e6	1.7e6	4.5e6	4.1e6	1.1e6	6.2e6	2.2e6
F	7.4e6	6.8e6	3.4e6	2.0e6	1.2e6	5.6e5	4.1e5	6.0e5	4.4e6	2.2e6	1.3e6	1.2e6
G	9.3e6	1.7e6	1.7e6	7.1e6	7.0e5	2.6e6	1.5e6	8.3e5	3.9e5	2.1e6	4.5e6	7.1e4
H	6.7e6	6.3e5	8.1e6	1.5e6	7.5e4	1.2e6	4.1e6	4.0e5	1.6e6	6.7e5	1.2e6	7.1e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

- Fluorescence
- Lm1 485, 535
- Slide(s) Ex1, Em1
- More Settings
- Shake Off
- ReadOrder Row
- Show Optimizer On
- PMT and Optics
- Integration Time 400 ms
- Read from Top
- Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:30 PM
 3/17/2014

Mean Temperature : 27 °C

Plate05

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.4e6	6.3e6	7.0e6	1.2e7	2.8e6	1.2e6	3.1e6	3.7e6	9.6e5	1.8e6	7.4e5	3.4e7
B	1.5e6	2.9e6	4.2e6	3.7e6	2.0e6	2.0e6	1.4e6	1.7e6	2.6e6	4.9e6	1.4e6	1.8e7
C	7.2e6	9.8e6	2.1e6	9.0e6	7.9e4	1.5e6	1.9e6	2.1e6	5.4e5	1.9e6	2.0e6	9.5e6
D	1.6e6	4.6e6	2.7e6	1.8e6	8.0e5	1.3e6	7.8e4	8.1e5	4.0e6	2.1e6	5.8e5	4.8e6
E	1.6e6	1.4e6	2.1e6	1.2e6	1.3e6	2.3e6	1.6e6	4.2e6	4.4e6	1.2e6	6.6e6	2.5e6
F	8.1e6	6.7e6	3.3e6	1.8e6	1.1e6	5.1e5	4.3e5	5.3e5	4.4e6	2.2e6	1.5e6	1.2e6
G	8.7e6	1.7e6	1.8e6	7.1e6	5.7e5	2.6e6	1.4e6	8.5e5	3.5e5	1.8e6	4.4e6	7.2e4
H	6.6e6	6.7e5	7.5e6	1.5e6	7.6e4	1.1e6	4.3e6	4.2e5	1.6e6	5.6e5	1.2e6	7.2e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:32 PM
 3/17/2014

Mean Temperature : 27 °C

Plate06

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.3e6	6.1e6	6.5e6	1.1e7	2.6e6	1.1e6	3.1e6	3.5e6	1.0e6	1.6e6	6.5e5	3.4e7
B	1.5e6	2.7e6	4.0e6	3.7e6	2.1e6	1.6e6	1.4e6	1.6e6	2.7e6	4.1e6	1.3e6	1.8e7
C	6.4e6	9.2e6	2.1e6	8.0e6	7.7e4	1.4e6	1.8e6	1.9e6	5.0e5	1.8e6	1.9e6	9.2e6
D	1.5e6	4.4e6	2.3e6	1.8e6	7.3e5	1.3e6	7.5e4	8.0e5	3.9e6	2.1e6	1.2e6	4.6e6
E	1.4e6	1.2e6	2.1e6	1.1e6	1.2e6	2.2e6	1.7e6	4.0e6	3.9e6	1.1e6	5.5e6	2.4e6
F	7.4e6	6.5e6	2.9e6	1.6e6	1.1e6	5.0e5	4.0e5	5.2e5	4.0e6	2.0e6	1.3e6	1.2e6
G	7.9e6	1.5e6	1.7e6	6.8e6	5.8e5	2.5e6	1.4e6	8.2e5	3.2e5	1.8e6	3.6e6	7.0e4
H	6.1e6	6.3e5	6.7e6	1.4e6	7.4e4	1.1e6	4.0e6	4.3e5	1.6e6	5.8e5	1.1e6	6.9e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:35 PM
 3/17/2014

Mean Temperature : 27 °C

Plate07

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.5e6	6.6e6	7.1e6	1.2e7	2.7e6	1.1e6	3.2e6	3.8e6	1.1e6	1.6e6	6.9e5	3.4e7
B	1.6e6	2.9e6	4.3e6	3.8e6	2.2e6	1.6e6	1.4e6	1.7e6	2.7e6	4.4e6	1.4e6	1.8e7
C	6.7e6	9.9e6	2.2e6	8.8e6	7.7e4	1.5e6	1.9e6	1.9e6	5.2e5	1.8e6	1.9e6	1.0e7
D	1.5e6	4.5e6	2.5e6	1.9e6	7.8e5	1.3e6	7.5e4	8.4e5	3.9e6	2.1e6	5.8e5	4.6e6
E	1.5e6	1.3e6	2.2e6	1.2e6	1.3e6	2.2e6	1.6e6	4.2e6	4.0e6	1.1e6	6.1e6	2.3e6
F	7.4e6	6.5e6	3.1e6	1.6e6	1.1e6	5.0e5	4.1e5	5.1e5	4.3e6	2.0e6	1.4e6	1.3e6
G	8.4e6	1.6e6	1.7e6	7.1e6	6.0e5	2.5e6	1.4e6	8.2e5	3.6e5	1.9e6	4.0e6	7.1e4
H	6.3e6	6.2e5	6.7e6	1.4e6	7.5e4	1.1e6	4.0e6	4.0e5	1.6e6	5.9e5	1.1e6	7.0e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:38 PM
 3/17/2014

Mean Temperature : 27.5 °C

Standards

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
01	100.000	170.999	A1	33448858.0...	36866650.000	29...	8.1
		199.885	A2	38808812.0...			
		197.371	A3	38342280.0...			
02	50.000	78.895	B1	16358536.0...	17064083.333	64...	3.8
		83.463	B2	17206134.0...			
		85.734	B3	17627580.0...			
03	25.000	37.338	C1	8647505.000	8863854.333	19...	2.2
		39.416	C2	9033007.000			
		38.759	C3	8911051.000			
04	12.500	14.287	D1	4370224.000	4399097.000	31...	0.7
		14.413	D2	4393706.000			
		14.627	D3	4433361.000			
05	6.250	1.575	E1	2011537.000	2101767.000	10...	5.2
		1.892	E2	2070325.000			
		2.717	E3	2223439.000			
06	3.125	-3.924	F1	991104.000	1065234.667	64...	6.0
		-3.299	F2	1107124.000			
		-3.351	F3	1097476.000			
07	0.000	-8.919	G1	64193.000	65239.000	15...	2.4
		-8.918	G2	64478.000			
		-8.904	G3	67046.000			
08	100.000	-8.927	H1	62813.000	64703.000	17...	2.7
		-8.914	H2	65134.000			
		-8.909	H3	66162.000			
09	0.000	-8.913	A10	65479.000	68842.708	11...	16.8
		-8.912	A11	65640.000			
		-8.915	A12	65056.000			
		-8.897	B10	68372.000			
		-8.905	B11	66873.000			
		-8.919	B12	64268.000			
		-8.904	C10	67087.000			
		-8.904	C11	66973.000			
		-8.604	C12	122816.000			
		-8.892	D10	69238.000			
		-8.905	D11	66814.000			
		-8.904	D12	67031.000			
-8.893	E10	69149.000					
-8.905	E11	66957.000					
-8.904	E12	67122.000					
-8.910	F10	66018.000					
-8.909	F11	66092.000					
-8.892	F12	69355.000					
-8.902	G10	67514.000					
-8.912	G11	65531.000					
-8.914	G12	65196.000					
-8.914	H10	65119.000					
-8.920	H11	64134.000					
-8.918	H12	64391.000					

Unknowns

Sample	Wells	RFU_Values	Concentration	Mean_Conc	SD	CV	Dilution	AdjConc
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Unknowns_NoDiln

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
01	A1	3554228.000	9.889	9.889	0.000	0.0
02	B1	1550118.000	-0.911	-0.911	0.000	0.0
03	C1	6512137.000	25.830	25.830	0.000	0.0
04	D1	1587653.000	-0.709	-0.709	0.000	0.0
05	E1	1571668.000	-0.795	-0.795	0.000	0.0
06	F1	7310432.000	30.132	30.132	0.000	0.0
07	G1	9208370.000	40.361	40.361	0.000	0.0
08	H1	6779996.000	27.274	27.274	0.000	0.0
09	A2	6314054.000	24.763	24.763	0.000	0.0
097	A1	3078374.000	7.325	7.325	0.000	0.0
098	B1	1399458.000	-1.723	-1.723	0.000	0.0
099	C1	5899655.000	22.529	22.529	0.000	0.0
10	B2	2901264.000	6.370	6.370	0.000	0.0
100	D1	1444820.000	-1.479	-1.479	0.000	0.0
101	E1	1431579.000	-1.550	-1.550	0.000	0.0
102	F1	5998115.000	23.060	23.060	0.000	0.0
103	G1	7298061.000	30.066	30.066	0.000	0.0
104	H1	5723340.000	21.579	21.579	0.000	0.0
105	A2	6009992.000	23.124	23.124	0.000	0.0
106	B2	2490836.000	4.158	4.158	0.000	0.0
107	C2	8658503.000	37.398	37.398	0.000	0.0
108	D2	5025297.000	17.817	17.817	0.000	0.0
109	E2	1184866.000	-2.880	-2.880	0.000	0.0
11	C2	10447351.000	47.038	47.038	0.000	0.0
110	F2	5795752.000	21.969	21.969	0.000	0.0
111	G2	1398738.000	-1.727	-1.727	0.000	0.0
112	H2	528585.000	-6.417	-6.417	0.000	0.0
113	A3	6319127.000	24.790	24.790	0.000	0.0
114	B3	4008962.000	12.340	12.340	0.000	0.0
115	C3	1937402.000	1.176	1.176	0.000	0.0
116	D3	2391934.000	3.625	3.625	0.000	0.0
117	E3	2218454.000	2.690	2.690	0.000	0.0
118	F3	3053427.000	7.190	7.190	0.000	0.0
119	G3	1613835.000	-0.568	-0.568	0.000	0.0
12	D2	5746318.000	21.703	21.703	0.000	0.0
120	H3	6917400.000	28.014	28.014	0.000	0.0
121	A4	9404010.000	41.415	41.415	0.000	0.0
122	B4	3502573.000	9.611	9.611	0.000	0.0
123	C4	8118685.000	34.488	34.488	0.000	0.0
124	D4	1934053.000	1.158	1.158	0.000	0.0
125	E4	1224272.000	-2.667	-2.667	0.000	0.0
126	F4	1831291.000	0.604	0.604	0.000	0.0
127	G4	6937371.000	28.122	28.122	0.000	0.0
128	H4	1450050.000	-1.451	-1.451	0.000	0.0
129	A5	2506091.000	4.241	4.241	0.000	0.0
13	E2	1384338.000	-1.805	-1.805	0.000	0.0
130	B5	1708154.000	-0.060	-0.060	0.000	0.0
131	C5	77221.000	-8.849	-8.849	0.000	0.0
132	D5	798343.000	-4.963	-4.963	0.000	0.0
133	E5	1352012.000	-1.979	-1.979	0.000	0.0
134	F5	1136712.000	-3.139	-3.139	0.000	0.0
135	G5	714592.000	-5.414	-5.414	0.000	0.0
136	H5	74227.000	-8.865	-8.865	0.000	0.0
137	A6	1178626.000	-2.913	-2.913	0.000	0.0
138	B6	1659333.000	-0.323	-0.323	0.000	0.0
139	C6	1445548.000	-1.475	-1.475	0.000	0.0
14	F2	6903892.000	27.941	27.941	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
140	D6	1294962.000	-2.287	-2.287	0.000	0.0
141	E6	2337784.000	3.334	3.334	0.000	0.0
142	F6	585773.000	-6.109	-6.109	0.000	0.0
143	G6	2640582.000	4.965	4.965	0.000	0.0
144	H6	1136594.000	-3.140	-3.140	0.000	0.0
145	A7	3040236.000	7.119	7.119	0.000	0.0
146	B7	1405063.000	-1.693	-1.693	0.000	0.0
147	C7	1863891.000	0.780	0.780	0.000	0.0
148	D7	75222.000	-8.860	-8.860	0.000	0.0
149	E7	1575254.000	-0.776	-0.776	0.000	0.0
15	G2	1759466.000	0.217	0.217	0.000	0.0
150	F7	379911.000	-7.218	-7.218	0.000	0.0
151	G7	1463127.000	-1.380	-1.380	0.000	0.0
152	H7	3871109.000	11.597	11.597	0.000	0.0
153	A8	3541074.000	9.818	9.818	0.000	0.0
154	B8	1718207.000	-0.006	-0.006	0.000	0.0
155	C8	2334449.000	3.316	3.316	0.000	0.0
156	D8	817260.000	-4.861	-4.861	0.000	0.0
157	E8	3974197.000	12.153	12.153	0.000	0.0
158	F8	581799.000	-6.130	-6.130	0.000	0.0
159	G8	799391.000	-4.957	-4.957	0.000	0.0
16	H2	645041.000	-5.789	-5.789	0.000	0.0
160	H8	405495.000	-7.080	-7.080	0.000	0.0
161	A9	1154234.000	-3.045	-3.045	0.000	0.0
162	B9	2404566.000	3.693	3.693	0.000	0.0
163	C9	577629.000	-6.152	-6.152	0.000	0.0
164	D9	3486331.000	9.523	9.523	0.000	0.0
165	E9	3819818.000	11.321	11.321	0.000	0.0
166	F9	4045998.000	12.540	12.540	0.000	0.0
167	G9	350397.000	-7.377	-7.377	0.000	0.0
168	H9	1540135.000	-0.965	-0.965	0.000	0.0
169	A10	1526532.000	-1.039	-1.039	0.000	0.0
17	A3	7313784.000	30.151	30.151	0.000	0.0
170	B10	4301127.000	13.915	13.915	0.000	0.0
171	C10	1700361.000	-0.102	-0.102	0.000	0.0
172	D10	2121458.000	2.168	2.168	0.000	0.0
173	E10	1044789.000	-3.635	-3.635	0.000	0.0
174	F10	1874273.000	0.836	0.836	0.000	0.0
175	G10	1806569.000	0.471	0.471	0.000	0.0
176	H10	573093.000	-6.177	-6.177	0.000	0.0
177	A11	677303.000	-5.615	-5.615	0.000	0.0
178	B11	1291321.000	-2.306	-2.306	0.000	0.0
179	C11	1948710.000	1.237	1.237	0.000	0.0
18	B3	4621651.000	15.642	15.642	0.000	0.0
180	D11	618204.000	-5.934	-5.934	0.000	0.0
181	E11	5508100.000	20.419	20.419	0.000	0.0
182	F11	1167153.000	-2.975	-2.975	0.000	0.0
183	G11	3707518.000	10.715	10.715	0.000	0.0
184	H11	1000524.000	-3.873	-3.873	0.000	0.0
185	A12	67884.000	-8.900	-8.900	0.000	0.0
186	B12	66184.000	-8.909	-8.909	0.000	0.0
187	C12	66843.000	-8.905	-8.905	0.000	0.0
188	D12	67234.000	-8.903	-8.903	0.000	0.0
189	E12	67343.000	-8.902	-8.902	0.000	0.0
19	C3	2338756.000	3.339	3.339	0.000	0.0
190	F12	67655.000	-8.901	-8.901	0.000	0.0
191	G12	66077.000	-8.909	-8.909	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
192	H12	64865.000	-8.916	-8.916	0....	0.0
193	A1	3808310.000	11.259	11.259	0....	0.0
194	B1	1679993.000	-0.211	-0.211	0....	0.0
195	C1	6873091.000	27.776	27.776	0....	0.0
196	D1	1696818.000	-0.121	-0.121	0....	0.0
197	E1	1542873.000	-0.950	-0.950	0....	0.0
198	F1	7412208.000	30.681	30.681	0....	0.0
199	G1	9340930.000	41.075	41.075	0....	0.0
20	D3	2838887.000	6.034	6.034	0....	0.0
200	H1	6659352.000	26.624	26.624	0....	0.0
201	A2	6561762.000	26.098	26.098	0....	0.0
202	B2	3022532.000	7.024	7.024	0....	0.0
203	C2	10549812.0...	47.590	47.590	0....	0.0
204	D2	5702673.000	21.468	21.468	0....	0.0
205	E2	1351540.000	-1.982	-1.982	0....	0.0
206	F2	6786715.000	27.310	27.310	0....	0.0
207	G2	1692387.000	-0.145	-0.145	0....	0.0
208	H2	627930.000	-5.881	-5.881	0....	0.0
209	A3	7409903.000	30.669	30.669	0....	0.0
21	E3	2534414.000	4.393	4.393	0....	0.0
210	B3	4452551.000	14.731	14.731	0....	0.0
211	C3	2334567.000	3.316	3.316	0....	0.0
212	D3	2459679.000	3.990	3.990	0....	0.0
213	E3	2357961.000	3.442	3.442	0....	0.0
214	F3	3385445.000	8.980	8.980	0....	0.0
215	G3	1650610.000	-0.370	-0.370	0....	0.0
216	H3	8074077.000	34.248	34.248	0....	0.0
217	A4	11611440.0...	53.312	53.312	0....	0.0
218	B4	3855596.000	11.513	11.513	0....	0.0
219	C4	9442131.000	41.621	41.621	0....	0.0
22	F3	3387535.000	8.991	8.991	0....	0.0
220	D4	2026692.000	1.657	1.657	0....	0.0
221	E4	1274150.000	-2.399	-2.399	0....	0.0
222	F4	2015970.000	1.599	1.599	0....	0.0
223	G4	7125144.000	29.134	29.134	0....	0.0
224	H4	1489085.000	-1.240	-1.240	0....	0.0
225	A5	2790782.000	5.775	5.775	0....	0.0
226	B5	1867627.000	0.800	0.800	0....	0.0
227	C5	78657.000	-8.841	-8.841	0....	0.0
228	D5	900968.000	-4.410	-4.410	0....	0.0
229	E5	1490646.000	-1.232	-1.232	0....	0.0
23	G3	1750851.000	0.170	0.170	0....	0.0
230	F5	1150903.000	-3.063	-3.063	0....	0.0
231	G5	702499.000	-5.479	-5.479	0....	0.0
232	H5	75079.000	-8.861	-8.861	0....	0.0
233	A6	1283548.000	-2.348	-2.348	0....	0.0
234	B6	1822647.000	0.557	0.557	0....	0.0
235	C6	1546744.000	-0.930	-0.930	0....	0.0
236	D6	1442325.000	-1.492	-1.492	0....	0.0
237	E6	2396531.000	3.650	3.650	0....	0.0
238	F6	560448.000	-6.245	-6.245	0....	0.0
239	G6	2640611.000	4.966	4.966	0....	0.0
24	H3	8618981.000	37.185	37.185	0....	0.0
240	H6	1174935.000	-2.933	-2.933	0....	0.0
241	A7	3234324.000	8.165	8.165	0....	0.0
242	B7	1427727.000	-1.571	-1.571	0....	0.0
243	C7	2098006.000	2.041	2.041	0....	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
244	D7	76704.000	-8.852	-8.852	0.000	0.00
245	E7	1747044.000	0.150	0.150	0.000	0.00
246	F7	406654.000	-7.074	-7.074	0.000	0.00
247	G7	1532652.000	-1.006	-1.006	0.000	0.00
248	H7	4064245.000	12.638	12.638	0.000	0.00
249	A8	3763024.000	11.015	11.015	0.000	0.00
25	A4	11915491.000	54.950	54.950	0.000	0.00
250	B8	1738792.000	0.105	0.105	0.000	0.00
251	C8	2566920.000	4.568	4.568	0.000	0.00
252	D8	906494.000	-4.380	-4.380	0.000	0.00
253	E8	4458872.000	14.765	14.765	0.000	0.00
254	F8	604444.000	-6.008	-6.008	0.000	0.00
255	G8	830171.000	-4.791	-4.791	0.000	0.00
256	H8	402299.000	-7.097	-7.097	0.000	0.00
257	A9	1266909.000	-2.438	-2.438	0.000	0.00
258	B9	2830222.000	5.987	5.987	0.000	0.00
259	C9	602274.000	-6.020	-6.020	0.000	0.00
26	B4	3890251.000	11.700	11.700	0.000	0.00
260	D9	3990990.000	12.243	12.243	0.000	0.00
261	E9	4139587.000	13.044	13.044	0.000	0.00
262	F9	4382925.000	14.355	14.355	0.000	0.00
263	G9	391841.000	-7.154	-7.154	0.000	0.00
264	H9	1625724.000	-0.504	-0.504	0.000	0.00
265	A10	1762401.000	0.233	0.233	0.000	0.00
266	B10	4705262.000	16.093	16.093	0.000	0.00
267	C10	1883945.000	0.888	0.888	0.000	0.00
268	D10	2269862.000	2.967	2.967	0.000	0.00
269	E10	1116850.000	-3.246	-3.246	0.000	0.00
27	C4	9535605.000	42.124	42.124	0.000	0.00
270	F10	2158209.000	2.366	2.366	0.000	0.00
271	G10	2064948.000	1.863	1.863	0.000	0.00
272	H10	667595.000	-5.668	-5.668	0.000	0.00
273	A11	710381.000	-5.437	-5.437	0.000	0.00
274	B11	1444392.000	-1.481	-1.481	0.000	0.00
275	C11	2128327.000	2.205	2.205	0.000	0.00
276	D11	652245.000	-5.750	-5.750	0.000	0.00
277	E11	6200665.000	24.152	24.152	0.000	0.00
278	F11	1328122.000	-2.108	-2.108	0.000	0.00
279	G11	4472370.000	14.837	14.837	0.000	0.00
28	D4	2123484.000	2.179	2.179	0.000	0.00
280	H11	1178097.000	-2.916	-2.916	0.000	0.00
281	A12	32387288.000	165.278	165.278	0.000	0.00
282	B12	16659357.000	80.516	80.516	0.000	0.00
283	C12	11132437.000	50.730	50.730	0.000	0.00
284	D12	4641974.000	15.751	15.751	0.000	0.00
285	E12	2240373.000	2.809	2.809	0.000	0.00
286	F12	1181087.000	-2.900	-2.900	0.000	0.00
287	G12	71040.000	-8.883	-8.883	0.000	0.00
288	H12	70689.000	-8.884	-8.884	0.000	0.00
289	A1	3417404.000	9.152	9.152	0.000	0.00
29	E4	1352857.000	-1.974	-1.974	0.000	0.00
290	B1	1546272.000	-0.932	-0.932	0.000	0.00
291	C1	7243901.000	29.774	29.774	0.000	0.00
292	D1	1583114.000	-0.734	-0.734	0.000	0.00
293	E1	1557656.000	-0.871	-0.871	0.000	0.00
294	F1	8144321.000	34.626	34.626	0.000	0.00
295	G1	8737072.000	37.821	37.821	0.000	0.00

Unknowns_NoDiIn (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
296	H1	6587768.000	26.238	26.238	0.000	0.00
297	A2	6307068.000	24.725	24.725	0.000	0.00
298	B2	2858406.000	6.139	6.139	0.000	0.00
299	C2	9764864.000	43.360	43.360	0.000	0.00
30	F4	2135413.000	2.243	2.243	0.000	0.00
300	D2	4634116.000	15.709	15.709	0.000	0.00
301	E2	1370980.000	-1.877	-1.877	0.000	0.00
302	F2	6749411.000	27.109	27.109	0.000	0.00
303	G2	1662902.000	-0.304	-0.304	0.000	0.00
304	H2	665964.000	-5.676	-5.676	0.000	0.00
305	A3	7024703.000	28.593	28.593	0.000	0.00
306	B3	4249264.000	13.635	13.635	0.000	0.00
307	C3	2098867.000	2.046	2.046	0.000	0.00
308	D3	2652435.000	5.029	5.029	0.000	0.00
309	E3	2138819.000	2.261	2.261	0.000	0.00
31	G4	7902314.000	33.322	33.322	0.000	0.00
310	F3	3269137.000	8.353	8.353	0.000	0.00
311	G3	1805938.000	0.467	0.467	0.000	0.00
312	H3	7464640.000	30.964	30.964	0.000	0.00
313	A4	12317143.000	57.115	57.115	0.000	0.00
314	B4	3711801.000	10.738	10.738	0.000	0.00
315	C4	9010063.000	39.292	39.292	0.000	0.00
316	D4	1838813.000	0.644	0.644	0.000	0.00
317	E4	1194625.000	-2.827	-2.827	0.000	0.00
318	F4	1784316.000	0.351	0.351	0.000	0.00
319	G4	7081948.000	28.901	28.901	0.000	0.00
32	H4	1467442.000	-1.357	-1.357	0.000	0.00
320	H4	1472290.000	-1.331	-1.331	0.000	0.00
321	A5	2793869.000	5.792	5.792	0.000	0.00
322	B5	2029391.000	1.672	1.672	0.000	0.00
323	C5	79158.000	-8.839	-8.839	0.000	0.00
324	D5	802161.000	-4.942	-4.942	0.000	0.00
325	E5	1291532.000	-2.305	-2.305	0.000	0.00
326	F5	1100088.000	-3.337	-3.337	0.000	0.00
327	G5	570123.000	-6.193	-6.193	0.000	0.00
328	H5	76306.000	-8.854	-8.854	0.000	0.00
329	A6	1216527.000	-2.709	-2.709	0.000	0.00
33	A5	2976749.000	6.777	6.777	0.000	0.00
330	B6	2049344.000	1.779	1.779	0.000	0.00
331	C6	1460454.000	-1.395	-1.395	0.000	0.00
332	D6	1340631.000	-2.040	-2.040	0.000	0.00
333	E6	2328752.000	3.285	3.285	0.000	0.00
334	F6	513468.000	-6.498	-6.498	0.000	0.00
335	G6	2649147.000	5.012	5.012	0.000	0.00
336	H6	1102425.000	-3.324	-3.324	0.000	0.00
337	A7	3122101.000	7.560	7.560	0.000	0.00
338	B7	1381289.000	-1.821	-1.821	0.000	0.00
339	C7	1890740.000	0.924	0.924	0.000	0.00
34	B5	1934063.000	1.158	1.158	0.000	0.00
340	D7	77787.000	-8.846	-8.846	0.000	0.00
341	E7	1632831.000	-0.466	-0.466	0.000	0.00
342	F7	431107.000	-6.942	-6.942	0.000	0.00
343	G7	1369292.000	-1.886	-1.886	0.000	0.00
344	H7	4347627.000	14.165	14.165	0.000	0.00
345	A8	3681653.000	10.576	10.576	0.000	0.00
346	B8	1740338.000	0.114	0.114	0.000	0.00
347	C8	2075675.000	1.921	1.921	0.000	0.00

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
348	D8	814981.000	-4.873	-4.873	0.000	0.0
349	E8	4214950.000	13.450	13.450	0.000	0.0
35	C5	78990.000	-8.840	-8.840	0.000	0.0
350	F8	525785.000	-6.432	-6.432	0.000	0.0
351	G8	849218.000	-4.689	-4.689	0.000	0.0
352	H8	424794.000	-6.976	-6.976	0.000	0.0
353	A9	962831.000	-4.076	-4.076	0.000	0.0
354	B9	2614560.000	4.825	4.825	0.000	0.0
355	C9	536003.000	-6.377	-6.377	0.000	0.0
356	D9	3961261.000	12.083	12.083	0.000	0.0
357	E9	4399430.000	14.444	14.444	0.000	0.0
358	F9	4378151.000	14.330	14.330	0.000	0.0
359	G9	352274.000	-7.367	-7.367	0.000	0.0
36	D5	966725.000	-4.055	-4.055	0.000	0.0
360	H9	1597752.000	-0.655	-0.655	0.000	0.0
361	A10	1810730.000	0.493	0.493	0.000	0.0
362	B10	4891757.000	17.098	17.098	0.000	0.0
363	C10	1886300.000	0.900	0.900	0.000	0.0
364	D10	2148028.000	2.311	2.311	0.000	0.0
365	E10	1153216.000	-3.050	-3.050	0.000	0.0
366	F10	2207762.000	2.633	2.633	0.000	0.0
367	G10	1813451.000	0.508	0.508	0.000	0.0
368	H10	555013.000	-6.274	-6.274	0.000	0.0
369	A11	744987.000	-5.250	-5.250	0.000	0.0
37	E5	1569560.000	-0.807	-0.807	0.000	0.0
370	B11	1387551.000	-1.788	-1.788	0.000	0.0
371	C11	2045376.000	1.758	1.758	0.000	0.0
372	D11	579120.000	-6.144	-6.144	0.000	0.0
373	E11	6566846.000	26.125	26.125	0.000	0.0
374	F11	1481816.000	-1.279	-1.279	0.000	0.0
375	G11	4371767.000	14.295	14.295	0.000	0.0
376	H11	1164049.000	-2.992	-2.992	0.000	0.0
377	A12	33830684.000	173.057	173.057	0.000	0.0
378	B12	18183756.000	88.732	88.732	0.000	0.0
379	C12	9546355.000	42.182	42.182	0.000	0.0
38	F5	1203390.000	-2.780	-2.780	0.000	0.0
380	D12	4757585.000	16.374	16.374	0.000	0.0
381	E12	2481572.000	4.108	4.108	0.000	0.0
382	F12	1237400.000	-2.597	-2.597	0.000	0.0
383	G12	72067.000	-8.877	-8.877	0.000	0.0
384	H12	71828.000	-8.878	-8.878	0.000	0.0
385	A1	3281543.000	8.420	8.420	0.000	0.0
386	B1	1455491.000	-1.421	-1.421	0.000	0.0
387	C1	6411782.000	25.289	25.289	0.000	0.0
388	D1	1475541.000	-1.313	-1.313	0.000	0.0
389	E1	1423690.000	-1.593	-1.593	0.000	0.0
39	G5	731300.000	-5.324	-5.324	0.000	0.0
390	F1	7351493.000	30.354	30.354	0.000	0.0
391	G1	7934940.000	33.498	33.498	0.000	0.0
392	H1	6055274.000	23.368	23.368	0.000	0.0
393	A2	6068248.000	23.438	23.438	0.000	0.0
394	B2	2745873.000	5.533	5.533	0.000	0.0
395	C2	9183855.000	40.229	40.229	0.000	0.0
396	D2	4351531.000	14.186	14.186	0.000	0.0
397	E2	1247318.000	-2.543	-2.543	0.000	0.0
398	F2	6498747.000	25.758	25.758	0.000	0.0
399	G2	1547786.000	-0.924	-0.924	0.000	0.0

Unknowns_NoDiIn (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
40	H5	75075.000	-8.861	-8.861	0.000	0.00
400	H2	625395.000	-5.895	-5.895	0.000	0.00
401	A3	6477227.000	25.642	25.642	0.000	0.00
402	B3	3971624.000	12.139	12.139	0.000	0.00
403	C3	2094304.000	2.021	2.021	0.000	0.00
404	D3	2329242.000	3.288	3.288	0.000	0.00
405	E3	2086400.000	1.979	1.979	0.000	0.00
406	F3	2919691.000	6.470	6.470	0.000	0.00
407	G3	1650270.000	-0.372	-0.372	0.000	0.00
408	H3	6693879.000	26.810	26.810	0.000	0.00
409	A4	11111554.000	50.618	50.618	0.000	0.00
41	A6	1314297.000	-2.182	-2.182	0.000	0.00
410	B4	3652486.000	10.419	10.419	0.000	0.00
411	C4	7962653.000	33.647	33.647	0.000	0.00
412	D4	1832976.000	0.613	0.613	0.000	0.00
413	E4	1120779.000	-3.225	-3.225	0.000	0.00
414	F4	1637924.000	-0.438	-0.438	0.000	0.00
415	G4	6848426.000	27.643	27.643	0.000	0.00
416	H4	1405896.000	-1.689	-1.689	0.000	0.00
417	A5	2630680.000	4.912	4.912	0.000	0.00
418	B5	2080377.000	1.946	1.946	0.000	0.00
419	C5	76854.000	-8.851	-8.851	0.000	0.00
42	B6	1969474.000	1.349	1.349	0.000	0.00
420	D5	729169.000	-5.336	-5.336	0.000	0.00
421	E5	1239261.000	-2.587	-2.587	0.000	0.00
422	F5	1088495.000	-3.399	-3.399	0.000	0.00
423	G5	576257.000	-6.160	-6.160	0.000	0.00
424	H5	73762.000	-8.868	-8.868	0.000	0.00
425	A6	1085563.000	-3.415	-3.415	0.000	0.00
426	B6	1578289.000	-0.760	-0.760	0.000	0.00
427	C6	1412768.000	-1.652	-1.652	0.000	0.00
428	D6	1286588.000	-2.332	-2.332	0.000	0.00
429	E6	2176459.000	2.464	2.464	0.000	0.00
43	C6	1633222.000	-0.464	-0.464	0.000	0.00
430	F6	500447.000	-6.568	-6.568	0.000	0.00
431	G6	2452672.000	3.953	3.953	0.000	0.00
432	H6	1086514.000	-3.410	-3.410	0.000	0.00
433	A7	3137822.000	7.645	7.645	0.000	0.00
434	B7	1351589.000	-1.981	-1.981	0.000	0.00
435	C7	1832045.000	0.608	0.608	0.000	0.00
436	D7	74645.000	-8.863	-8.863	0.000	0.00
437	E7	1681981.000	-0.201	-0.201	0.000	0.00
438	F7	402974.000	-7.094	-7.094	0.000	0.00
439	G7	1352267.000	-1.978	-1.978	0.000	0.00
44	D6	1489365.000	-1.239	-1.239	0.000	0.00
440	H7	3956843.000	12.059	12.059	0.000	0.00
441	A8	3540361.000	9.815	9.815	0.000	0.00
442	B8	1635308.000	-0.452	-0.452	0.000	0.00
443	C8	1857310.000	0.744	0.744	0.000	0.00
444	D8	797743.000	-4.966	-4.966	0.000	0.00
445	E8	3971570.000	12.138	12.138	0.000	0.00
446	F8	517876.000	-6.474	-6.474	0.000	0.00
447	G8	816798.000	-4.863	-4.863	0.000	0.00
448	H8	429862.000	-6.949	-6.949	0.000	0.00
449	A9	1026440.000	-3.734	-3.734	0.000	0.00
45	E6	2482128.000	4.111	4.111	0.000	0.00
450	B9	2690799.000	5.236	5.236	0.000	0.00

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
451	C9	504683.000	-6.546	-6.546	0.000	0.00
452	D9	3854135.000	11.506	11.506	0.000	0.00
453	E9	3892002.000	11.710	11.710	0.000	0.00
454	F9	4047475.000	12.548	12.548	0.000	0.00
455	G9	322640.000	-7.527	-7.527	0.000	0.00
456	H9	1604697.000	-0.617	-0.617	0.000	0.00
457	A10	1579227.000	-0.755	-0.755	0.000	0.00
458	B10	4120469.000	12.941	12.941	0.000	0.00
459	C10	1799047.000	0.430	0.430	0.000	0.00
46	F6	594054.000	-6.064	-6.064	0.000	0.00
460	D10	2052425.000	1.796	1.796	0.000	0.00
461	E10	1117822.000	-3.241	-3.241	0.000	0.00
462	F10	1977228.000	1.390	1.390	0.000	0.00
463	G10	1801444.000	0.443	0.443	0.000	0.00
464	H10	583513.000	-6.121	-6.121	0.000	0.00
465	A11	646640.000	-5.780	-5.780	0.000	0.00
466	B11	1300600.000	-2.256	-2.256	0.000	0.00
467	C11	1887159.000	0.905	0.905	0.000	0.00
468	D11	1210443.000	-2.742	-2.742	0.000	0.00
469	E11	5484091.000	20.290	20.290	0.000	0.00
47	G6	2724039.000	5.415	5.415	0.000	0.00
470	F11	1344731.000	-2.018	-2.018	0.000	0.00
471	G11	3633717.000	10.318	10.318	0.000	0.00
472	H11	1139194.000	-3.126	-3.126	0.000	0.00
473	A12	33580628.000	171.709	171.709	0.000	0.00
474	B12	17933646.000	87.384	87.384	0.000	0.00
475	C12	9219952.000	40.423	40.423	0.000	0.00
476	D12	4580124.000	15.418	15.418	0.000	0.00
477	E12	2418428.000	3.768	3.768	0.000	0.00
478	F12	1193556.000	-2.833	-2.833	0.000	0.00
479	G12	69751.000	-8.889	-8.889	0.000	0.00
48	H6	1138300.000	-3.131	-3.131	0.000	0.00
480	H12	68728.000	-8.895	-8.895	0.000	0.00
481	A1	3487629.000	9.530	9.530	0.000	0.00
482	B1	1563380.000	-0.840	-0.840	0.000	0.00
483	C1	6743788.000	27.079	27.079	0.000	0.00
484	D1	1545879.000	-0.934	-0.934	0.000	0.00
485	E1	1524728.000	-1.048	-1.048	0.000	0.00
486	F1	7376833.000	30.490	30.490	0.000	0.00
487	G1	8447563.000	36.261	36.261	0.000	0.00
488	H1	6296497.000	24.668	24.668	0.000	0.00
489	A2	6572096.000	26.153	26.153	0.000	0.00
49	A7	3439830.000	9.273	9.273	0.000	0.00
490	B2	2879062.000	6.251	6.251	0.000	0.00
491	C2	9898176.000	44.078	44.078	0.000	0.00
492	D2	4464962.000	14.797	14.797	0.000	0.00
493	E2	1306968.000	-2.222	-2.222	0.000	0.00
494	F2	6495845.000	25.742	25.742	0.000	0.00
495	G2	1581453.000	-0.743	-0.743	0.000	0.00
496	H2	624398.000	-5.900	-5.900	0.000	0.00
497	A3	7131496.000	29.168	29.168	0.000	0.00
498	B3	4345328.000	14.153	14.153	0.000	0.00
499	C3	2213941.000	2.666	2.666	0.000	0.00
50	B7	1511477.000	-1.120	-1.120	0.000	0.00
500	D3	2473268.000	4.064	4.064	0.000	0.00
501	E3	2193317.000	2.555	2.555	0.000	0.00
502	F3	3111756.000	7.505	7.505	0.000	0.00

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
503	G3	1729020.000	0.053	0.053	0....	0.0
504	H3	6673714.000	26.701	26.701	0....	0.0
505	A4	11574284.0...	53.111	53.111	0....	0.0
506	B4	3815883.000	11.299	11.299	0....	0.0
507	C4	8810389.000	38.216	38.216	0....	0.0
508	D4	1890500.000	0.923	0.923	0....	0.0
509	E4	1180565.000	-2.903	-2.903	0....	0.0
51	C7	2205605.000	2.621	2.621	0....	0.0
510	F4	1637601.000	-0.440	-0.440	0....	0.0
511	G4	7063621.000	28.802	28.802	0....	0.0
512	H4	1400839.000	-1.716	-1.716	0....	0.0
513	A5	2689121.000	5.227	5.227	0....	0.0
514	B5	2180877.000	2.488	2.488	0....	0.0
515	C5	77349.000	-8.849	-8.849	0....	0.0
516	D5	776120.000	-5.083	-5.083	0....	0.0
517	E5	1252824.000	-2.514	-2.514	0....	0.0
518	F5	1119228.000	-3.234	-3.234	0....	0.0
519	G5	598217.000	-6.041	-6.041	0....	0.0
52	D7	76770.000	-8.852	-8.852	0....	0.0
520	H5	75123.000	-8.861	-8.861	0....	0.0
521	A6	1146925.000	-3.084	-3.084	0....	0.0
522	B6	1622522.000	-0.521	-0.521	0....	0.0
523	C6	1491899.000	-1.225	-1.225	0....	0.0
524	D6	1315526.000	-2.176	-2.176	0....	0.0
525	E6	2196790.000	2.574	2.574	0....	0.0
526	F6	504981.000	-6.544	-6.544	0....	0.0
527	G6	2487171.000	4.139	4.139	0....	0.0
528	H6	1132269.000	-3.163	-3.163	0....	0.0
529	A7	3245045.000	8.223	8.223	0....	0.0
53	E7	1825860.000	0.575	0.575	0....	0.0
530	B7	1383015.000	-1.812	-1.812	0....	0.0
531	C7	1912680.000	1.043	1.043	0....	0.0
532	D7	74707.000	-8.863	-8.863	0....	0.0
533	E7	1577768.000	-0.762	-0.762	0....	0.0
534	F7	414755.000	-7.030	-7.030	0....	0.0
535	G7	1412001.000	-1.656	-1.656	0....	0.0
536	H7	3957064.000	12.060	12.060	0....	0.0
537	A8	3758371.000	10.989	10.989	0....	0.0
538	B8	1704704.000	-0.078	-0.078	0....	0.0
539	C8	1908384.000	1.019	1.019	0....	0.0
54	F7	436701.000	-6.912	-6.912	0....	0.0
540	D8	836832.000	-4.755	-4.755	0....	0.0
541	E8	4232899.000	13.547	13.547	0....	0.0
542	F8	507753.000	-6.529	-6.529	0....	0.0
543	G8	818469.000	-4.854	-4.854	0....	0.0
544	H8	395961.000	-7.131	-7.131	0....	0.0
545	A9	1071994.000	-3.488	-3.488	0....	0.0
546	B9	2716508.000	5.375	5.375	0....	0.0
547	C9	516960.000	-6.479	-6.479	0....	0.0
548	D9	3948148.000	12.012	12.012	0....	0.0
549	E9	3994769.000	12.263	12.263	0....	0.0
55	G7	1579687.000	-0.752	-0.752	0....	0.0
550	F9	4342670.000	14.138	14.138	0....	0.0
551	G9	356708.000	-7.343	-7.343	0....	0.0
552	H9	1642793.000	-0.412	-0.412	0....	0.0
553	A10	1635868.000	-0.449	-0.449	0....	0.0
554	B10	4447590.000	14.704	14.704	0....	0.0

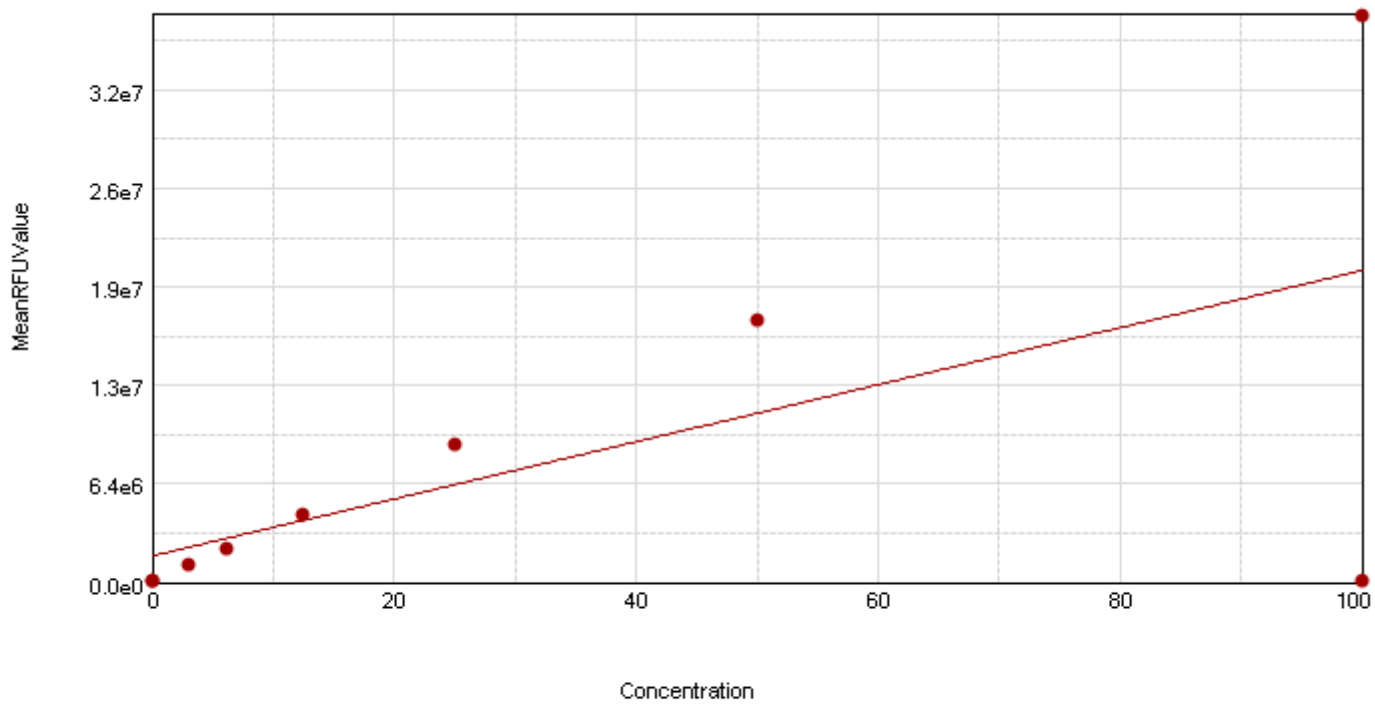
Unknowns_NoDiIn (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
555	C10	1802882.000	0.451	0.451	0....	0.0
556	D10	2095185.000	2.026	2.026	0....	0.0
557	E10	1115917.000	-3.251	-3.251	0....	0.0
558	F10	2024019.000	1.643	1.643	0....	0.0
559	G10	1903850.000	0.995	0.995	0....	0.0
56	H7	4274919.000	13.773	13.773	0....	0.0
560	H10	592462.000	-6.072	-6.072	0....	0.0
561	A11	688453.000	-5.555	-5.555	0....	0.0
562	B11	1367940.000	-1.893	-1.893	0....	0.0
563	C11	1903260.000	0.992	0.992	0....	0.0
564	D11	584022.000	-6.118	-6.118	0....	0.0
565	E11	6113651.000	23.683	23.683	0....	0.0
566	F11	1356446.000	-1.955	-1.955	0....	0.0
567	G11	3957068.000	12.060	12.060	0....	0.0
568	H11	1122054.000	-3.218	-3.218	0....	0.0
569	A12	33542128.0...	171.502	171.502	0....	0.0
57	A8	4073236.000	12.686	12.686	0....	0.0
570	B12	17732026.0...	86.297	86.297	0....	0.0
571	C12	10087986.0...	45.101	45.101	0....	0.0
572	D12	4634229.000	15.710	15.710	0....	0.0
573	E12	2315433.000	3.213	3.213	0....	0.0
574	F12	1259960.000	-2.475	-2.475	0....	0.0
575	G12	70794.000	-8.884	-8.884	0....	0.0
576	H12	69949.000	-8.888	-8.888	0....	0.0
577	A4	2680142.000	5.179	5.179	0....	0.0
578	B4	1412534.000	-1.653	-1.653	0....	0.0
579	C4	2768345.000	5.654	5.654	0....	0.0
58	B8	1909254.000	1.024	1.024	0....	0.0
580	D4	2303778.000	3.150	3.150	0....	0.0
581	E4	852189.000	-4.673	-4.673	0....	0.0
582	F4	2001796.000	1.523	1.523	0....	0.0
583	G4	509714.000	-6.518	-6.518	0....	0.0
584	H4	771154.000	-5.109	-5.109	0....	0.0
585	A5	2709957.000	5.339	5.339	0....	0.0
586	B5	1732240.000	0.070	0.070	0....	0.0
587	C5	3026048.000	7.043	7.043	0....	0.0
588	D5	2755305.000	5.584	5.584	0....	0.0
589	E5	1019310.000	-3.772	-3.772	0....	0.0
59	C8	2694219.000	5.254	5.254	0....	0.0
590	F5	2499401.000	4.205	4.205	0....	0.0
591	G5	651679.000	-5.753	-5.753	0....	0.0
592	H5	730056.000	-5.331	-5.331	0....	0.0
593	A6	3027630.000	7.051	7.051	0....	0.0
594	B6	1803196.000	0.453	0.453	0....	0.0
595	C6	3024412.000	7.034	7.034	0....	0.0
596	D6	2844395.000	6.064	6.064	0....	0.0
597	E6	1092798.000	-3.376	-3.376	0....	0.0
598	F6	2414725.000	3.748	3.748	0....	0.0
599	G6	660785.000	-5.704	-5.704	0....	0.0
60	D8	947690.000	-4.158	-4.158	0....	0.0
600	H6	767668.000	-5.128	-5.128	0....	0.0
601	A7	548923.000	-6.307	-6.307	0....	0.0
602	B7	1847395.000	0.691	0.691	0....	0.0
603	C7	3264216.000	8.326	8.326	0....	0.0
604	D7	2841354.000	6.047	6.047	0....	0.0
605	E7	1128512.000	-3.184	-3.184	0....	0.0
606	F7	2701752.000	5.295	5.295	0....	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
607	G7	585345.000	-6.111	-6.111	0.000	0.0
608	H7	676451.000	-5.620	-5.620	0.000	0.0
609	A8	573408.000	-6.175	-6.175	0.000	0.0
61	E8	4569142.000	15.359	15.359	0.000	0.0
610	B8	1880843.000	0.871	0.871	0.000	0.0
611	C8	3251456.000	8.258	8.258	0.000	0.0
612	D8	2906525.000	6.399	6.399	0.000	0.0
613	E8	1085921.000	-3.413	-3.413	0.000	0.0
614	F8	2676092.000	5.157	5.157	0.000	0.0
615	G8	609453.000	-5.981	-5.981	0.000	0.0
616	H8	695230.000	-5.519	-5.519	0.000	0.0
617	A9	531919.000	-6.399	-6.399	0.000	0.0
618	B9	1645077.000	-0.400	-0.400	0.000	0.0
619	C9	2988179.000	6.839	6.839	0.000	0.0
62	F8	641806.000	-5.807	-5.807	0.000	0.0
620	D9	2623402.000	4.873	4.873	0.000	0.0
621	E9	1110972.000	-3.278	-3.278	0.000	0.0
622	F9	2411829.000	3.733	3.733	0.000	0.0
623	G9	560757.000	-6.243	-6.243	0.000	0.0
624	H9	644190.000	-5.794	-5.794	0.000	0.0
63	G8	870304.000	-4.575	-4.575	0.000	0.0
64	H8	432474.000	-6.935	-6.935	0.000	0.0
65	A9	1332128.000	-2.086	-2.086	0.000	0.0
66	B9	2893044.000	6.326	6.326	0.000	0.0
67	C9	661893.000	-5.698	-5.698	0.000	0.0
68	D9	4115344.000	12.913	12.913	0.000	0.0
69	E9	4360300.000	14.233	14.233	0.000	0.0
70	F9	4659844.000	15.848	15.848	0.000	0.0
71	G9	392096.000	-7.152	-7.152	0.000	0.0
72	H9	1743758.000	0.132	0.132	0.000	0.0
73	A10	1802850.000	0.451	0.451	0.000	0.0
74	B10	5021469.000	17.797	17.797	0.000	0.0
75	C10	1977290.000	1.391	1.391	0.000	0.0
76	D10	2367764.000	3.495	3.495	0.000	0.0
77	E10	1157864.000	-3.025	-3.025	0.000	0.0
78	F10	2306068.000	3.163	3.163	0.000	0.0
79	G10	2120771.000	2.164	2.164	0.000	0.0
80	H10	684518.000	-5.576	-5.576	0.000	0.0
81	A11	756049.000	-5.191	-5.191	0.000	0.0
82	B11	1523817.000	-1.053	-1.053	0.000	0.0
83	C11	2327899.000	3.280	3.280	0.000	0.0
84	D11	703624.000	-5.473	-5.473	0.000	0.0
85	E11	6521884.000	25.883	25.883	0.000	0.0
86	F11	1408839.000	-1.673	-1.673	0.000	0.0
87	G11	4636840.000	15.724	15.724	0.000	0.0
88	H11	1286458.000	-2.332	-2.332	0.000	0.0
89	A12	71753.000	-8.879	-8.879	0.000	0.0
90	B12	72277.000	-8.876	-8.876	0.000	0.0
91	C12	72254.000	-8.876	-8.876	0.000	0.0
92	D12	71850.000	-8.878	-8.878	0.000	0.0
93	E12	72283.000	-8.876	-8.876	0.000	0.0
94	F12	77726.000	-8.847	-8.847	0.000	0.0
95	G12	186201.000	-8.262	-8.262	0.000	0.0
96	H12	69032.000	-8.893	-8.893	0.000	0.0

Standard Curve



● STD#1 (Standards@PicoGreen: MeanRF... vs Concentr...)

Curve Fit Results ▼

Intro

Quantitation of double-stranded DNA using Quant-iT PicoGreen Reagent

Invitrogen (Molecular Probes)

MATERIALS

- o Quant-iT PicoGreen dsDNA Assay Kit, including lambda DNA standard (Invitrogen cat. #P7589 or P11496)
- o Black 96-well plate (Greiner Bio-One, cat. # 655096)
- o Brown or amber (light-blocking) microcentrifuge tubes

METHODS

Set up the protocol:

- o Select Wells to Read and Assay Plate Type by clicking on "Settings" and locating the options on the left side of the screen.
- o Click the Template button to open a window where you can assign wells of the microplate to pre-set template groups using the drop-down menu to select the appropriate template group. There are preconfigured template groups in the PicoGreen Fluorescence protocol including Standards, Unknowns, and Unknowns_NoDiln (for undiluted samples). Assigning wells to pre-set template groups populates group tables in the protocol with the corresponding data acquired when the microplate is read.

Prepare the assay

The method for this assay follows the instructions in the product information sheet for Quant-iT PicoGreen dsDNA Reagent and Kits from Molecular Probes, except that the assay volume is proportionately reduced from 2.0 mL to 200 μ L to fit a 96-well microplate format.

- o Prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) by diluting the concentrated buffer from the kit 20-fold with distilled DNase-free water, as required by Molecular Probes.
- o Prepare an aqueous working solution of Quant-iT PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE buffer (prepared above). Preparation of the solution in a plastic container, rather than glass, is recommended, as the reagent may adsorb to glass surfaces. Protect the solution from light by using amber or brown tubes, or by covering with foil. This solution should be used within a few hours of its preparation.
- o DNA standard curve: Prepare a 2 μ g/mL stock solution of dsDNA in TE. The lambda DNA standard provided with the kit can be diluted 50-fold in TE to make the 2 μ g/mL solution. Note: in some cases it may be preferable to make the standard curve using DNA similar to the type being assayed.
- o A high-range standard curve may be prepared from 1 ng/mL to 1 μ g/mL, or a low-range standard curve may be prepared from 25 μ g/mL to 25 ng/mL. For the high-range curve, follow the dilution scheme shown in the PicoGreen product insert; for the low-range curve, dilute the 2 μ g/mL solution 40-fold to yield a 50 ng/mL solution, and refer to the alternative dilution scheme in the product insert.
- o Pipet standards into a solid black 96-well microplate at 100 μ L per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).
- o Add 100 μ L of the aqueous working solution of Quant-iT PicoGreen reagent to each well. Mix well by trituration or plate shaker and incubate for 2 to 5 minutes at room temperature, protected from light.

Read the microplate

- " Make sure the purple plate adapter is in the microplate reader drawer. Place the microplate in the drawer.
- " Click the Read button in the SoftMax Pro software. The instrument will read the plate and the relative fluorescence units will be displayed in the Plate section of the protocol.

Analyze the data

- o After the microplate has been read, the relative fluorescence units (RFUs) will be displayed in the Plate section. The data will be analyzed in the Group Tables that were created when the template was set up.
- o Standards assigned in the Template (and thus displayed in the Standards group table) will be automatically plotted in the Standard Curve section of the protocol. A linear curve fit is applied by default, but a log-log fit may be used when plotting a standard curve over a wide dynamic range. Curve fits are chosen from the drop-down Curve Fit menu in the graph section's tool bar.

READER SUITABILITY:

All SpectraMax readers with fluorescence capability.

PROTOCOL REVISION HISTORY:

- v 1.1: Imported from SMP 5.4.2 April 2011 (CLO & ELM)
- v 1.2: Emission wavelength changed from 540 nm to 525 nm. (CLO)

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.4e7	3.9e7	3.7e7	2.5e6	2.6e6	2.9e6	5.3e5	5.5e5	5.1e5	6.4e4	6.3e4	6.4e4
B	1.6e7	1.6e7	1.7e7	1.3e6	1.6e6	1.7e6	1.7e6	1.8e6	1.5e6	6.6e4	6.4e4	6.3e4
C	8.3e6	8.6e6	8.4e6	2.6e6	2.9e6	2.9e6	3.1e6	3.1e6	2.8e6	6.5e4	6.4e4	1.3e5
D	4.1e6	4.1e6	4.1e6	2.2e6	2.6e6	2.7e6	2.7e6	2.7e6	2.5e6	6.6e4	6.4e4	6.5e4
E	1.9e6	1.9e6	2.1e6	8.1e5	9.6e5	1.0e6	1.1e6	1.0e6	1.0e6	6.6e4	6.5e4	6.5e4
F	9.4e5	1.0e6	1.0e6	1.9e6	2.4e6	2.3e6	2.5e6	2.5e6	2.3e6	6.4e4	6.4e4	6.7e4
G	6.4e4	6.4e4	6.4e4	4.8e5	8.2e5	6.3e5	5.6e5	5.8e5	5.4e5	8.1e4	6.5e4	6.3e4
H	6.3e4	6.4e4	6.4e4	7.7e5	7.0e5	7.4e5	6.5e5	6.6e5	6.2e5	6.4e4	6.3e4	6.3e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint
 Fluorescence
 Lm1 485,535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:51 PM
 3/17/2014

Mean Temperature : 28.5 °C

Plate02

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.5e6	6.1e6	7.0e6	1.1e7	2.8e6	1.3e6	3.3e6	3.8e6	1.3e6	1.7e6	7.2e5	7.0e4
B	1.5e6	2.8e6	4.4e6	3.7e6	1.8e6	1.9e6	1.4e6	1.8e6	2.8e6	4.7e6	1.5e6	7.0e4
C	6.3e6	1.0e7	2.2e6	9.0e6	7.7e4	1.5e6	2.1e6	2.6e6	6.2e5	1.9e6	2.1e6	7.1e4
D	1.5e6	5.5e6	2.7e6	2.0e6	9.1e5	1.4e6	7.5e4	9.1e5	3.8e6	2.2e6	6.5e5	7.1e4
E	1.5e6	1.3e6	2.4e6	1.3e6	1.5e6	2.3e6	1.7e6	4.4e6	4.1e6	1.1e6	6.2e6	7.1e4
F	7.2e6	6.6e6	3.2e6	2.0e6	1.1e6	5.6e5	4.2e5	6.1e5	4.4e6	2.1e6	1.3e6	7.1e4
G	8.9e6	1.7e6	1.7e6	7.6e6	7.0e5	2.6e6	1.5e6	8.3e5	3.7e5	2.0e6	4.4e6	1.9e5
H	6.6e6	6.2e5	8.3e6	1.4e6	7.5e4	1.1e6	4.0e6	4.1e5	1.7e6	6.6e5	1.2e6	6.8e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint
 Fluorescence
 Lm1 485,535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:53 PM
 3/17/2014

Mean Temperature : 28.5 °C

Plate03

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.9e6	5.8e6	6.1e6	9.1e6	2.4e6	1.1e6	2.9e6	3.4e6	1.1e6	1.4e6	7.5e5	6.7e4
B	1.4e6	2.4e6	3.8e6	3.4e6	1.6e6	1.6e6	1.3e6	1.6e6	2.3e6	4.1e6	1.3e6	6.6e4
C	5.7e6	8.6e6	1.9e6	8.0e6	7.6e4	1.4e6	1.8e6	2.3e6	5.6e5	1.6e6	1.9e6	6.6e4
D	1.4e6	4.8e6	2.3e6	1.9e6	7.6e5	1.3e6	7.1e4	7.9e5	3.3e6	2.1e6	6.0e5	6.7e4
E	1.4e6	1.1e6	2.1e6	1.2e6	1.3e6	2.3e6	1.5e6	3.9e6	3.7e6	1.0e6	5.4e6	6.8e4
F	5.8e6	5.6e6	2.9e6	1.7e6	1.1e6	5.6e5	3.7e5	5.6e5	3.9e6	1.8e6	1.1e6	6.7e4
G	7.2e6	1.3e6	1.6e6	6.7e6	6.9e5	2.6e6	1.4e6	7.8e5	3.4e5	1.8e6	3.6e6	6.6e4
H	5.5e6	5.0e5	6.8e6	1.4e6	7.2e4	1.1e6	3.7e6	4.0e5	1.5e6	5.6e5	9.8e5	6.5e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint
 Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:56 PM
 3/17/2014

Mean Temperature : 28.5 °C

Plate04

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.6e6	6.2e6	7.0e6	1.1e7	2.6e6	1.2e6	3.1e6	3.5e6	1.2e6	1.7e6	6.7e5	3.3e7
B	1.6e6	2.9e6	4.2e6	3.7e6	1.8e6	1.7e6	1.4e6	1.7e6	2.7e6	4.5e6	1.4e6	1.6e7
C	6.5e6	1.0e7	2.2e6	8.9e6	7.6e4	1.5e6	2.0e6	2.5e6	5.8e5	1.8e6	2.0e6	1.0e7
D	1.6e6	5.4e6	2.3e6	1.9e6	8.5e5	1.4e6	7.4e4	8.7e5	3.8e6	2.2e6	6.2e5	4.3e6
E	1.5e6	1.3e6	2.2e6	1.2e6	1.4e6	2.3e6	1.6e6	4.2e6	4.0e6	1.1e6	5.9e6	2.1e6
F	6.9e6	6.5e6	3.2e6	1.9e6	1.1e6	5.3e5	3.9e5	5.8e5	4.2e6	2.0e6	1.3e6	1.1e6
G	8.8e6	1.6e6	1.6e6	6.8e6	6.7e5	2.5e6	1.5e6	7.9e5	3.7e5	1.9e6	4.2e6	6.8e4
H	6.2e6	5.8e5	7.6e6	1.4e6	7.3e4	1.1e6	3.8e6	3.8e5	1.5e6	6.2e5	1.1e6	6.8e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint
 Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 2:59 PM
 3/17/2014

Mean Temperature : 28.5 °C

Plate05

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.2e6	6.0e6	6.5e6	1.2e7	2.6e6	1.2e6	3.0e6	3.4e6	9.1e5	1.7e6	7.0e5	3.3e7
B	1.4e6	2.7e6	4.0e6	3.5e6	1.9e6	1.9e6	1.3e6	1.6e6	2.5e6	4.6e6	1.3e6	1.7e7
C	6.8e6	9.3e6	2.0e6	8.5e6	7.5e4	1.4e6	1.8e6	2.0e6	5.1e5	1.8e6	1.9e6	8.7e6
D	1.5e6	4.4e6	2.5e6	1.7e6	7.6e5	1.3e6	7.5e4	7.8e5	3.7e6	2.0e6	5.4e5	4.3e6
E	1.5e6	1.3e6	2.0e6	1.1e6	1.2e6	2.2e6	1.5e6	4.0e6	4.1e6	1.1e6	6.1e6	2.3e6
F	7.6e6	6.4e6	3.1e6	1.7e6	1.0e6	4.8e5	4.1e5	5.0e5	4.1e6	2.0e6	1.4e6	1.1e6
G	8.3e6	1.5e6	1.7e6	6.7e6	5.4e5	2.5e6	1.3e6	8.0e5	3.4e5	1.7e6	4.1e6	6.9e4
H	6.2e6	6.2e5	7.1e6	1.4e6	7.3e4	1.0e6	4.1e6	4.0e5	1.5e6	5.2e5	1.1e6	6.9e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

- Fluorescence
- Lm1 485,535
- Slide(s) Ex1, Em1
- More Settings
- Shake Off
- ReadOrder Row
- Show Optimizer On
- PMT and Optics
- Integration Time 400 ms
- Read from Top
- Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:02 PM
 3/17/2014

Mean Temperature : 28.5 °C

Plate06

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.0e6	5.7e6	6.1e6	1.1e7	2.5e6	1.0e6	3.0e6	3.3e6	9.6e5	1.5e6	6.1e5	3.4e7
B	1.4e6	2.6e6	3.8e6	3.5e6	2.0e6	1.5e6	1.3e6	1.5e6	2.5e6	3.9e6	1.2e6	1.7e7
C	6.1e6	8.7e6	2.0e6	7.6e6	7.5e4	1.3e6	1.7e6	1.8e6	4.8e5	1.7e6	1.8e6	8.5e6
D	1.4e6	4.1e6	2.2e6	1.7e6	6.9e5	1.2e6	7.2e4	7.6e5	3.6e6	2.0e6	1.0e6	4.1e6
E	1.4e6	1.2e6	2.0e6	1.1e6	1.2e6	2.0e6	1.7e6	3.8e6	3.7e6	1.1e6	5.2e6	2.1e6
F	7.0e6	6.2e6	2.8e6	1.5e6	1.0e6	4.7e5	3.9e5	4.9e5	3.8e6	1.8e6	1.3e6	1.1e6
G	7.5e6	1.4e6	1.6e6	6.5e6	5.5e5	2.3e6	1.3e6	8.0e5	3.1e5	1.7e6	3.4e6	6.7e4
H	5.7e6	5.9e5	6.3e6	1.3e6	7.1e4	1.0e6	3.7e6	4.1e5	1.6e6	5.4e5	1.1e6	6.7e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

- Fluorescence
- Lm1 485,535
- Slide(s) Ex1, Em1
- More Settings
- Shake Off
- ReadOrder Row
- Show Optimizer On
- PMT and Optics
- Integration Time 400 ms
- Read from Top
- Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:04 PM
 3/17/2014

Mean Temperature : 28.5 °C

Plate07

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.3e6	6.2e6	6.7e6	1.1e7	2.5e6	1.1e6	3.1e6	3.5e6	1.0e6	1.5e6	6.5e5	3.3e7
B	1.5e6	2.8e6	4.1e6	3.6e6	2.1e6	1.5e6	1.3e6	1.6e6	2.6e6	4.1e6	1.3e6	1.7e7
C	6.5e6	9.4e6	2.0e6	8.3e6	7.5e4	1.4e6	1.8e6	1.8e6	5.0e5	1.7e6	1.8e6	9.5e6
D	1.5e6	4.2e6	2.3e6	1.8e6	7.4e5	1.3e6	1.1e5	7.9e5	3.7e6	2.0e6	5.5e5	4.3e6
E	1.5e6	1.2e6	2.1e6	1.1e6	1.2e6	2.1e6	1.5e6	4.0e6	3.8e6	1.1e6	5.7e6	2.2e6
F	6.9e6	6.1e6	2.9e6	1.5e6	1.1e6	4.8e5	3.9e5	4.8e5	4.0e6	1.9e6	1.3e6	1.2e6
G	8.0e6	1.5e6	1.6e6	6.7e6	5.7e5	2.4e6	1.4e6	7.8e5	3.4e5	1.8e6	3.7e6	6.9e4
H	5.9e6	5.8e5	6.4e6	1.3e6	7.3e4	1.1e6	3.7e6	3.7e5	1.6e6	5.6e5	1.1e6	6.8e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

- Fluorescence
- Lm1 485, 535
- Slide(s) Ex1, Em1
- More Settings
- Shake Off
- ReadOrder Row
- Show Optimizer On
- PMT and Optics
- Integration Time 400 ms
- Read from Top
- Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:07 PM
 3/17/2014

Mean Temperature : 29 °C

Standards

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
01	100.000	158.647	A1	34321460.0...	36857716.000	24...	6.5
		181.055	A2	39116656.0...			
		171.795	A3	37135032.0...			
02	50.000	73.067	B1	16007753.0...	16409938.333	38...	2.3
		75.141	B2	16451509.0...			
		76.632	B3	16770553.0...			
03	25.000	37.005	C1	8290652.000	8439260.667	14...	1.7
		38.354	C2	8579295.000			
		37.740	C3	8447835.000			
04	12.500	17.519	D1	4120562.000	4111152.000	81...	0.2
		17.451	D2	4106108.000			
		17.454	D3	4106786.000			
05	6.250	7.104	E1	1891914.000	1974114.333	10...	5.3
		7.321	E2	1938434.000			
		8.039	E3	2091995.000			
06	3.125	2.634	F1	935281.000	1003025.667	59...	5.9
		3.142	F2	1043981.000			
		3.075	F3	1029815.000			
07	0.000	-1.440	G1	63621.000	63866.667	22...	0.3
		-1.438	G2	63932.000			
		-1.438	G3	64047.000			
08	100.000	-1.444	H1	62728.000	63539.667	70...	1.1
		-1.438	H2	63857.000			
		-1.438	H3	64034.000			
09	0.000	-1.439	A10	63715.000	67765.208	13...	19.4
		-1.441	A11	63216.000			
		-1.437	A12	64149.000			
		-1.428	B10	66057.000			
		-1.436	B11	64468.000			
		-1.443	B12	62941.000			
		-1.431	C10	65373.000			
		-1.438	C11	63959.000			
		-1.142	C12	127252.000			
		-1.426	D10	66441.000			
		-1.439	D11	63664.000			
		-1.433	D12	65089.000			
-1.428	E10	66187.000					
-1.433	E11	65007.000					
-1.433	E12	65065.000					
-1.438	F10	63878.000					
-1.438	F11	64003.000					
-1.422	F12	67469.000					
-1.359	G10	80809.000					
-1.434	G11	64895.000					
-1.442	G12	63083.000					
-1.439	H10	63684.000					
-1.444	H11	62591.000					
-1.441	H12	63370.000					
10	100.000	-1.410	A12	69869.000	69869.000	0....	0.0
11	50.000	-1.408	B12	70404.000	70404.000	0....	0.0
12	25.000	-1.406	C12	70807.000	70807.000	0....	0.0
13	12.500	-1.406	D12	70787.000	70787.000	0....	0.0
14	6.250	-1.405	E12	70925.000	70925.000	0....	0.0
15	3.125	-1.403	F12	71462.000	71462.000	0....	0.0
16	0.000	-0.862	G12	187274.000	187274.000	0....	0.0
17	0.000	-1.420	H12	67882.000	67882.000	0....	0.0
18	100.000	-1.426	A12	66519.000	66519.000	0....	0.0
19	50.000	-1.431	B12	65520.000	65520.000	0....	0.0
20	25.000	-1.428	C12	66189.000	66189.000	0....	0.0

Standards (Contd)

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
21	12.500	-1.425	D12	66661.000	66661.000	0....	0.0
22	6.250	-1.421	E12	67639.000	67639.000	0....	0.0
23	3.125	-1.424	F12	67001.000	67001.000	0....	0.0
24	0.000	-1.430	G12	65590.000	65590.000	0....	0.0
25	0.000	-1.432	H12	65260.000	65260.000	0....	0.0
26	100.000	151.224	A12	32732968.0...	32732968.000	0....	0.0
27	50.000	71.783	B12	15732990.0...	15732990.000	0....	0.0
28	25.000	47.221	C12	10476860.0...	10476860.000	0....	0.0
29	12.500	18.340	D12	4296320.000	4296320.000	0....	0.0
30	6.250	7.986	E12	2080705.000	2080705.000	0....	0.0
31	3.125	3.386	F12	1096282.000	1096282.000	0....	0.0
32	0.000	-1.420	G12	67830.000	67830.000	0....	0.0
33	0.000	-1.421	H12	67534.000	67534.000	0....	0.0
34	100.000	154.058	A12	33339470.0...	33339470.000	0....	0.0
35	50.000	77.390	B12	16932824.0...	16932824.000	0....	0.0
36	25.000	39.028	C12	8723497.000	8723497.000	0....	0.0
37	12.500	18.547	D12	4340571.000	4340571.000	0....	0.0
38	6.250	8.893	E12	2274786.000	2274786.000	0....	0.0
39	3.125	3.580	F12	1137721.000	1137721.000	0....	0.0
40	0.000	-1.415	G12	68849.000	68849.000	0....	0.0
41	0.000	-1.413	H12	69286.000	69286.000	0....	0.0
42	100.000	157.757	A12	34131056.0...	34131056.000	0....	0.0
43	50.000	77.441	B12	16943654.0...	16943654.000	0....	0.0
44	25.000	37.949	C12	8492656.000	8492656.000	0....	0.0
45	12.500	17.621	D12	4142526.000	4142526.000	0....	0.0
46	6.250	8.227	E12	2132268.000	2132268.000	0....	0.0
47	3.125	3.386	F12	1096286.000	1096286.000	0....	0.0
48	0.000	-1.422	G12	67266.000	67266.000	0....	0.0
49	0.000	-1.424	H12	66952.000	66952.000	0....	0.0
50	100.000	152.367	A12	32977576.0...	32977576.000	0....	0.0
51	50.000	76.001	B12	16635506.0...	16635506.000	0....	0.0
52	25.000	42.443	C12	9454297.000	9454297.000	0....	0.0
53	12.500	18.132	D12	4251915.000	4251915.000	0....	0.0
54	6.250	8.395	E12	2168225.000	2168225.000	0....	0.0
55	3.125	3.692	F12	1161827.000	1161827.000	0....	0.0
56	0.000	-1.414	G12	68981.000	68981.000	0....	0.0
57	0.000	-1.418	H12	68315.000	68315.000	0....	0.0

Unknowns

Sample	Wells	RFU_Values	Concentration	Mean_Conc	SD	CV	Dilution	AdjConc
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Unknowns_NoDiln

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
01	A1	3467187.000	14.465	14.465	0.000	0.0
02	B1	1502916.000	5.286	5.286	0.000	0.0
03	C1	6339339.000	27.887	27.887	0.000	0.0
04	D1	1549504.000	5.504	5.504	0.000	0.0
05	E1	1522894.000	5.380	5.380	0.000	0.0
06	F1	7196895.000	31.894	31.894	0.000	0.0
07	G1	8892387.000	39.817	39.817	0.000	0.0
08	H1	6557225.000	28.905	28.905	0.000	0.0
09	A2	6121597.000	26.869	26.869	0.000	0.0
097	A1	2942735.000	12.015	12.015	0.000	0.0
098	B1	1350913.000	4.576	4.576	0.000	0.0
099	C1	5656363.000	24.695	24.695	0.000	0.0
10	B2	2791573.000	11.308	11.308	0.000	0.0
100	D1	1391379.000	4.765	4.765	0.000	0.0
101	E1	1379759.000	4.711	4.711	0.000	0.0
102	F1	5750827.000	25.137	25.137	0.000	0.0
103	G1	7189401.000	31.859	31.859	0.000	0.0
104	H1	5512919.000	24.025	24.025	0.000	0.0
105	A2	5754329.000	25.153	25.153	0.000	0.0
106	B2	2408714.000	9.519	9.519	0.000	0.0
107	C2	8578169.000	38.349	38.349	0.000	0.0
108	D2	4808077.000	20.731	20.731	0.000	0.0
109	E2	1133396.000	3.560	3.560	0.000	0.0
11	C2	10036896.000	45.165	45.165	0.000	0.0
110	F2	5598632.000	24.426	24.426	0.000	0.0
111	G2	1342547.000	4.537	4.537	0.000	0.0
112	H2	500563.000	0.602	0.602	0.000	0.0
113	A3	6093354.000	26.737	26.737	0.000	0.0
114	B3	3838765.000	16.202	16.202	0.000	0.0
115	C3	1869232.000	6.998	6.998	0.000	0.0
116	D3	2305360.000	9.036	9.036	0.000	0.0
117	E3	2145240.000	8.288	8.288	0.000	0.0
118	F3	2945649.000	12.028	12.028	0.000	0.0
119	G3	1565272.000	5.578	5.578	0.000	0.0
12	D2	5513522.000	24.028	24.028	0.000	0.0
120	H3	6814610.000	30.108	30.108	0.000	0.0
121	A4	9120777.000	40.884	40.884	0.000	0.0
122	B4	3359359.000	13.961	13.961	0.000	0.0
123	C4	7998504.000	35.640	35.640	0.000	0.0
124	D4	1859320.000	6.952	6.952	0.000	0.0
125	E4	1158049.000	3.675	3.675	0.000	0.0
126	F4	1749634.000	6.439	6.439	0.000	0.0
127	G4	6719264.000	29.662	29.662	0.000	0.0
128	H4	1394403.000	4.779	4.779	0.000	0.0
129	A5	2412352.000	9.536	9.536	0.000	0.0
13	E2	1311562.000	4.392	4.392	0.000	0.0
130	B5	1640098.000	5.927	5.927	0.000	0.0
131	C5	75689.000	-1.383	-1.383	0.000	0.0
132	D5	763127.000	1.829	1.829	0.000	0.0
133	E5	1296475.000	4.322	4.322	0.000	0.0
134	F5	1088695.000	3.351	3.351	0.000	0.0
135	G5	693421.000	1.504	1.504	0.000	0.0
136	H5	72178.000	-1.400	-1.400	0.000	0.0
137	A6	1122976.000	3.511	3.511	0.000	0.0
138	B6	1595319.000	5.718	5.718	0.000	0.0
139	C6	1391390.000	4.765	4.765	0.000	0.0
14	F2	6628983.000	29.240	29.240	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
140	D6	1252341.000	4.115	4.115	0.000	0.00
141	E6	2264148.000	8.844	8.844	0.000	0.00
142	F6	563392.000	0.896	0.896	0.000	0.00
143	G6	2558064.000	10.217	10.217	0.000	0.00
144	H6	1100935.000	3.408	3.408	0.000	0.00
145	A7	2921379.000	11.915	11.915	0.000	0.00
146	B7	1340260.000	4.526	4.526	0.000	0.00
147	C7	1772862.000	6.548	6.548	0.000	0.00
148	D7	71497.000	-1.403	-1.403	0.000	0.00
149	E7	1508972.000	5.315	5.315	0.000	0.00
15	G2	1669247.000	6.064	6.064	0.000	0.00
150	F7	369064.000	-0.012	-0.012	0.000	0.00
151	G7	1425620.000	4.925	4.925	0.000	0.00
152	H7	3716734.000	15.631	15.631	0.000	0.00
153	A8	3448478.000	14.378	14.378	0.000	0.00
154	B8	1635082.000	5.904	5.904	0.000	0.00
155	C8	2270987.000	8.875	8.875	0.000	0.00
156	D8	789339.000	1.952	1.952	0.000	0.00
157	E8	3862153.000	16.311	16.311	0.000	0.00
158	F8	563036.000	0.894	0.894	0.000	0.00
159	G8	775192.000	1.886	1.886	0.000	0.00
16	H2	620853.000	1.164	1.164	0.000	0.00
160	H8	399634.000	0.131	0.131	0.000	0.00
161	A9	1099952.000	3.403	3.403	0.000	0.00
162	B9	2304646.000	9.033	9.033	0.000	0.00
163	C9	555932.000	0.861	0.861	0.000	0.00
164	D9	3345017.000	13.894	13.894	0.000	0.00
165	E9	3678325.000	15.452	15.452	0.000	0.00
166	F9	3917933.000	16.572	16.572	0.000	0.00
167	G9	340558.000	-0.145	-0.145	0.000	0.00
168	H9	1492479.000	5.238	5.238	0.000	0.00
169	A10	1448528.000	5.032	5.032	0.000	0.00
17	A3	7004420.000	30.995	30.995	0.000	0.00
170	B10	4086581.000	17.360	17.360	0.000	0.00
171	C10	1623428.000	5.849	5.849	0.000	0.00
172	D10	2053082.000	7.857	7.857	0.000	0.00
173	E10	1010068.000	2.983	2.983	0.000	0.00
174	F10	1785115.000	6.605	6.605	0.000	0.00
175	G10	1781369.000	6.587	6.587	0.000	0.00
176	H10	556759.000	0.865	0.865	0.000	0.00
177	A11	753347.000	1.784	1.784	0.000	0.00
178	B11	1255756.000	4.131	4.131	0.000	0.00
179	C11	1863771.000	6.973	6.973	0.000	0.00
18	B3	4376055.000	18.712	18.712	0.000	0.00
180	D11	596585.000	1.051	1.051	0.000	0.00
181	E11	5410965.000	23.549	23.549	0.000	0.00
182	F11	1129651.000	3.542	3.542	0.000	0.00
183	G11	3625189.000	15.204	15.204	0.000	0.00
184	H11	977968.000	2.833	2.833	0.000	0.00
19	C3	2207045.000	8.577	8.577	0.000	0.00
193	A1	3587432.000	15.027	15.027	0.000	0.00
194	B1	1586416.000	5.676	5.676	0.000	0.00
195	C1	6497889.000	28.628	28.628	0.000	0.00
196	D1	1592282.000	5.704	5.704	0.000	0.00
197	E1	1469585.000	5.131	5.131	0.000	0.00
198	F1	6941441.000	30.700	30.700	0.000	0.00
199	G1	8779279.000	39.289	39.289	0.000	0.00

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
20	D3	2686052.000	10.815	10.815	0.000	0.00
200	H1	6241906.000	27.432	27.432	0.000	0.00
201	A2	6194342.000	27.209	27.209	0.000	0.00
202	B2	2883549.000	11.738	11.738	0.000	0.00
203	C2	10112208.000	45.517	45.517	0.000	0.00
204	D2	5447232.000	23.718	23.718	0.000	0.00
205	E2	1277036.000	4.231	4.231	0.000	0.00
206	F2	6471067.000	28.502	28.502	0.000	0.00
207	G2	1559099.000	5.549	5.549	0.000	0.00
208	H2	583204.000	0.988	0.988	0.000	0.00
209	A3	6972400.000	30.845	30.845	0.000	0.00
21	E3	2427948.000	9.609	9.609	0.000	0.00
210	B3	4179667.000	17.795	17.795	0.000	0.00
211	C3	2199525.000	8.542	8.542	0.000	0.00
212	D3	2313429.000	9.074	9.074	0.000	0.00
213	E3	2249760.000	8.776	8.776	0.000	0.00
214	F3	3226190.000	13.339	13.339	0.000	0.00
215	G3	1570305.000	5.601	5.601	0.000	0.00
216	H3	7634530.000	33.939	33.939	0.000	0.00
217	A4	10767241.000	48.578	48.578	0.000	0.00
218	B4	3673416.000	15.429	15.429	0.000	0.00
219	C4	8946170.000	40.069	40.069	0.000	0.00
22	F3	3242880.000	13.417	13.417	0.000	0.00
220	D4	1908614.000	7.182	7.182	0.000	0.00
221	E4	1204769.000	3.893	3.893	0.000	0.00
222	F4	1911515.000	7.196	7.196	0.000	0.00
223	G4	6827616.000	30.169	30.169	0.000	0.00
224	H4	1384999.000	4.735	4.735	0.000	0.00
225	A5	2638292.000	10.592	10.592	0.000	0.00
226	B5	1787224.000	6.615	6.615	0.000	0.00
227	C5	76308.000	-1.380	-1.380	0.000	0.00
228	D5	849475.000	2.233	2.233	0.000	0.00
229	E5	1427719.000	4.935	4.935	0.000	0.00
23	G3	1682271.000	6.124	6.124	0.000	0.00
230	F5	1093985.000	3.375	3.375	0.000	0.00
231	G5	667647.000	1.383	1.383	0.000	0.00
232	H5	72902.000	-1.396	-1.396	0.000	0.00
233	A6	1226798.000	3.996	3.996	0.000	0.00
234	B6	1745318.000	6.419	6.419	0.000	0.00
235	C6	1458653.000	5.079	5.079	0.000	0.00
236	D6	1371609.000	4.673	4.673	0.000	0.00
237	E6	2277096.000	8.904	8.904	0.000	0.00
238	F6	531146.000	0.745	0.745	0.000	0.00
239	G6	2513779.000	10.010	10.010	0.000	0.00
24	H3	8294668.000	37.024	37.024	0.000	0.00
240	H6	1103616.000	3.420	3.420	0.000	0.00
241	A7	3066662.000	12.594	12.594	0.000	0.00
242	B7	1369511.000	4.663	4.663	0.000	0.00
243	C7	1962783.000	7.435	7.435	0.000	0.00
244	D7	73693.000	-1.392	-1.392	0.000	0.00
245	E7	1629077.000	5.876	5.876	0.000	0.00
246	F7	390053.000	0.086	0.086	0.000	0.00
247	G7	1465997.000	5.114	5.114	0.000	0.00
248	H7	3815503.000	16.093	16.093	0.000	0.00
249	A8	3546393.000	14.835	14.835	0.000	0.00
25	A4	11375547.000	51.421	51.421	0.000	0.00
250	B8	1653998.000	5.992	5.992	0.000	0.00

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
251	C8	2492998.000	9.913	9.913	0.000	0.0
252	D8	870740.000	2.332	2.332	0.000	0.0
253	E8	4227013.000	18.016	18.016	0.000	0.0
254	F8	580971.000	0.978	0.978	0.000	0.0
255	G8	790884.000	1.959	1.959	0.000	0.0
256	H8	375533.000	0.018	0.018	0.000	0.0
257	A9	1198299.000	3.863	3.863	0.000	0.0
258	B9	2689983.000	10.833	10.833	0.000	0.0
259	C9	581053.000	0.978	0.978	0.000	0.0
26	B4	3682678.000	15.472	15.472	0.000	0.0
260	D9	3788486.000	15.967	15.967	0.000	0.0
261	E9	3957017.000	16.754	16.754	0.000	0.0
262	F9	4158568.000	17.696	17.696	0.000	0.0
263	G9	374477.000	0.013	0.013	0.000	0.0
264	H9	1520153.000	5.367	5.367	0.000	0.0
265	A10	1669479.000	6.065	6.065	0.000	0.0
266	B10	4466058.000	19.133	19.133	0.000	0.0
267	C10	1806734.000	6.706	6.706	0.000	0.0
268	D10	2189674.000	8.496	8.496	0.000	0.0
269	E10	1066508.000	3.247	3.247	0.000	0.0
27	C4	9011406.000	40.373	40.373	0.000	0.0
270	F10	1977187.000	7.503	7.503	0.000	0.0
271	G10	1944834.000	7.351	7.351	0.000	0.0
272	H10	618852.000	1.155	1.155	0.000	0.0
273	A11	674635.000	1.416	1.416	0.000	0.0
274	B11	1386028.000	4.740	4.740	0.000	0.0
275	C11	2021934.000	7.712	7.712	0.000	0.0
276	D11	619087.000	1.156	1.156	0.000	0.0
277	E11	5919106.000	25.923	25.923	0.000	0.0
278	F11	1263447.000	4.167	4.167	0.000	0.0
279	G11	4243609.000	18.094	18.094	0.000	0.0
28	D4	1991409.000	7.569	7.569	0.000	0.0
280	H11	1106011.000	3.432	3.432	0.000	0.0
289	A1	3187927.000	13.160	13.160	0.000	0.0
29	E4	1278942.000	4.240	4.240	0.000	0.0
290	B1	1444995.000	5.016	5.016	0.000	0.0
291	C1	6831953.000	30.189	30.189	0.000	0.0
292	D1	1471212.000	5.138	5.138	0.000	0.0
293	E1	1466257.000	5.115	5.115	0.000	0.0
294	F1	7593846.000	33.749	33.749	0.000	0.0
295	G1	8271959.000	36.918	36.918	0.000	0.0
296	H1	6191490.000	27.196	27.196	0.000	0.0
297	A2	5951534.000	26.075	26.075	0.000	0.0
298	B2	2719433.000	10.971	10.971	0.000	0.0
299	C2	9257277.000	41.522	41.522	0.000	0.0
30	F4	2025083.000	7.726	7.726	0.000	0.0
300	D2	4354974.000	18.614	18.614	0.000	0.0
301	E2	1280335.000	4.246	4.246	0.000	0.0
302	F2	6391540.000	28.131	28.131	0.000	0.0
303	G2	1545897.000	5.487	5.487	0.000	0.0
304	H2	622567.000	1.172	1.172	0.000	0.0
305	A3	6494476.000	28.612	28.612	0.000	0.0
306	B3	3974183.000	16.834	16.834	0.000	0.0
307	C3	1954914.000	7.398	7.398	0.000	0.0
308	D3	2489819.000	9.898	9.898	0.000	0.0
309	E3	2010749.000	7.659	7.659	0.000	0.0
31	G4	7553005.000	33.558	33.558	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
310	F3	3090996.000	12.707	12.707	0.000	0.00
311	G3	1718153.000	6.292	6.292	0.000	0.00
312	H3	7087150.000	31.381	31.381	0.000	0.00
313	A4	11694361.000	52.911	52.911	0.000	0.00
314	B4	3509357.000	14.662	14.662	0.000	0.00
315	C4	8518561.000	38.070	38.070	0.000	0.00
316	D4	1706121.000	6.236	6.236	0.000	0.00
317	E4	1124413.000	3.518	3.518	0.000	0.00
318	F4	1674765.000	6.089	6.089	0.000	0.00
319	G4	6696606.000	29.556	29.556	0.000	0.00
32	H4	1418618.000	4.892	4.892	0.000	0.00
320	H4	1377000.000	4.698	4.698	0.000	0.00
321	A5	2628385.000	10.546	10.546	0.000	0.00
322	B5	1928616.000	7.276	7.276	0.000	0.00
323	C5	75352.000	-1.385	-1.385	0.000	0.00
324	D5	758145.000	1.806	1.806	0.000	0.00
325	E5	1214260.000	3.937	3.937	0.000	0.00
326	F5	1035631.000	3.103	3.103	0.000	0.00
327	G5	542649.000	0.799	0.799	0.000	0.00
328	H5	73433.000	-1.394	-1.394	0.000	0.00
329	A6	1151895.000	3.646	3.646	0.000	0.00
33	A5	2800766.000	11.351	11.351	0.000	0.00
330	B6	1942951.000	7.343	7.343	0.000	0.00
331	C6	1374722.000	4.687	4.687	0.000	0.00
332	D6	1275895.000	4.225	4.225	0.000	0.00
333	E6	2155064.000	8.334	8.334	0.000	0.00
334	F6	483742.000	0.524	0.524	0.000	0.00
335	G6	2499962.000	9.945	9.945	0.000	0.00
336	H6	1040654.000	3.126	3.126	0.000	0.00
337	A7	2951312.000	12.055	12.055	0.000	0.00
338	B7	1298025.000	4.329	4.329	0.000	0.00
339	C7	1761920.000	6.497	6.497	0.000	0.00
34	B5	1822453.000	6.779	6.779	0.000	0.00
340	D7	75181.000	-1.385	-1.385	0.000	0.00
341	E7	1530602.000	5.416	5.416	0.000	0.00
342	F7	411114.000	0.184	0.184	0.000	0.00
343	G7	1301715.000	4.346	4.346	0.000	0.00
344	H7	4062088.000	17.245	17.245	0.000	0.00
345	A8	3446780.000	14.370	14.370	0.000	0.00
346	B8	1639551.000	5.925	5.925	0.000	0.00
347	C8	1998787.000	7.603	7.603	0.000	0.00
348	D8	776835.000	1.893	1.893	0.000	0.00
349	E8	3977013.000	16.848	16.848	0.000	0.00
35	C5	76576.000	-1.379	-1.379	0.000	0.00
350	F8	502753.000	0.613	0.613	0.000	0.00
351	G8	804955.000	2.025	2.025	0.000	0.00
352	H8	400340.000	0.134	0.134	0.000	0.00
353	A9	907217.000	2.503	2.503	0.000	0.00
354	B9	2458418.000	9.751	9.751	0.000	0.00
355	C9	508029.000	0.637	0.637	0.000	0.00
356	D9	3662967.000	15.380	15.380	0.000	0.00
357	E9	4082556.000	17.341	17.341	0.000	0.00
358	F9	4084695.000	17.351	17.351	0.000	0.00
359	G9	336666.000	-0.164	-0.164	0.000	0.00
36	D5	908491.000	2.509	2.509	0.000	0.00
360	H9	1502840.000	5.286	5.286	0.000	0.00
361	A10	1699264.000	6.204	6.204	0.000	0.00

Unknowns_NoDiIn (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
362	B10	4578289.000	19.657	19.657	0.000	0.00
363	C10	1778695.000	6.575	6.575	0.000	0.00
364	D10	2041370.000	7.802	7.802	0.000	0.00
365	E10	1078786.000	3.304	3.304	0.000	0.00
366	F10	2025424.000	7.728	7.728	0.000	0.00
367	G10	1712202.000	6.264	6.264	0.000	0.00
368	H10	518768.000	0.687	0.687	0.000	0.00
369	A11	700462.000	1.536	1.536	0.000	0.00
37	E5	1485799.000	5.206	5.206	0.000	0.00
370	B11	1313361.000	4.401	4.401	0.000	0.00
371	C11	1916514.000	7.219	7.219	0.000	0.00
372	D11	542043.000	0.796	0.796	0.000	0.00
373	E11	6122426.000	26.873	26.873	0.000	0.00
374	F11	1388569.000	4.752	4.752	0.000	0.00
375	G11	4079775.000	17.328	17.328	0.000	0.00
376	H11	1088880.000	3.352	3.352	0.000	0.00
38	F5	1132877.000	3.557	3.557	0.000	0.00
385	A1	3042790.000	12.482	12.482	0.000	0.00
386	B1	1361007.000	4.623	4.623	0.000	0.00
387	C1	6071106.000	26.633	26.633	0.000	0.00
388	D1	1382078.000	4.722	4.722	0.000	0.00
389	E1	1366026.000	4.647	4.647	0.000	0.00
39	G5	698435.000	1.527	1.527	0.000	0.00
390	F1	6989735.000	30.926	30.926	0.000	0.00
391	G1	7546373.000	33.527	33.527	0.000	0.00
392	H1	5704069.000	24.918	24.918	0.000	0.00
393	A2	5711488.000	24.953	24.953	0.000	0.00
394	B2	2624725.000	10.528	10.528	0.000	0.00
395	C2	8725755.000	39.039	39.039	0.000	0.00
396	D2	4077536.000	17.317	17.317	0.000	0.00
397	E2	1175866.000	3.758	3.758	0.000	0.00
398	F2	6206768.000	27.267	27.267	0.000	0.00
399	G2	1446835.000	5.024	5.024	0.000	0.00
40	H5	74754.000	-1.387	-1.387	0.000	0.00
400	H2	585300.000	0.998	0.998	0.000	0.00
401	A3	6064604.000	26.603	26.603	0.000	0.00
402	B3	3769545.000	15.878	15.878	0.000	0.00
403	C3	1976388.000	7.499	7.499	0.000	0.00
404	D3	2193868.000	8.515	8.515	0.000	0.00
405	E3	1954084.000	7.395	7.395	0.000	0.00
406	F3	2758340.000	11.153	11.153	0.000	0.00
407	G3	1575217.000	5.624	5.624	0.000	0.00
408	H3	6341305.000	27.896	27.896	0.000	0.00
409	A4	10521686.000	47.431	47.431	0.000	0.00
41	A6	1253160.000	4.119	4.119	0.000	0.00
410	B4	3477798.000	14.515	14.515	0.000	0.00
411	C4	7574358.000	33.658	33.658	0.000	0.00
412	D4	1686439.000	6.144	6.144	0.000	0.00
413	E4	1050035.000	3.170	3.170	0.000	0.00
414	F4	1545392.000	5.485	5.485	0.000	0.00
415	G4	6464588.000	28.472	28.472	0.000	0.00
416	H4	1312438.000	4.396	4.396	0.000	0.00
417	A5	2479150.000	9.848	9.848	0.000	0.00
418	B5	1975258.000	7.494	7.494	0.000	0.00
419	C5	74733.000	-1.388	-1.388	0.000	0.00
42	B6	1889098.000	7.091	7.091	0.000	0.00
420	D5	694547.000	1.509	1.509	0.000	0.00

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
421	E5	1170688.000	3.734	3.734	0.000	0.00
422	F5	1024452.000	3.050	3.050	0.000	0.00
423	G5	553222.000	0.848	0.848	0.000	0.00
424	H5	71048.000	-1.405	-1.405	0.000	0.00
425	A6	1035058.000	3.100	3.100	0.000	0.00
426	B6	1496389.000	5.256	5.256	0.000	0.00
427	C6	1334547.000	4.500	4.500	0.000	0.00
428	D6	1217149.000	3.951	3.951	0.000	0.00
429	E6	2049838.000	7.842	7.842	0.000	0.00
43	C6	1549054.000	5.502	5.502	0.000	0.00
430	F6	474972.000	0.483	0.483	0.000	0.00
431	G6	2321686.000	9.112	9.112	0.000	0.00
432	H6	1021808.000	3.038	3.038	0.000	0.00
433	A7	2973352.000	12.158	12.158	0.000	0.00
434	B7	1282810.000	4.258	4.258	0.000	0.00
435	C7	1713442.000	6.270	6.270	0.000	0.00
436	D7	71785.000	-1.401	-1.401	0.000	0.00
437	E7	1672813.000	6.080	6.080	0.000	0.00
438	F7	385688.000	0.065	0.065	0.000	0.00
439	G7	1291642.000	4.299	4.299	0.000	0.00
44	D6	1408331.000	4.844	4.844	0.000	0.00
440	H7	3733757.000	15.711	15.711	0.000	0.00
441	A8	3310905.000	13.735	13.735	0.000	0.00
442	B8	1539486.000	5.457	5.457	0.000	0.00
443	C8	1797800.000	6.664	6.664	0.000	0.00
444	D8	761509.000	1.822	1.822	0.000	0.00
445	E8	3785291.000	15.952	15.952	0.000	0.00
446	F8	486295.000	0.536	0.536	0.000	0.00
447	G8	797581.000	1.990	1.990	0.000	0.00
448	H8	413051.000	0.193	0.193	0.000	0.00
449	A9	964665.000	2.771	2.771	0.000	0.00
45	E6	2344536.000	9.219	9.219	0.000	0.00
450	B9	2528295.000	10.078	10.078	0.000	0.00
451	C9	483650.000	0.523	0.523	0.000	0.00
452	D9	3586857.000	15.025	15.025	0.000	0.00
453	E9	3669605.000	15.411	15.411	0.000	0.00
454	F9	3825145.000	16.138	16.138	0.000	0.00
455	G9	305346.000	-0.310	-0.310	0.000	0.00
456	H9	1566258.000	5.582	5.582	0.000	0.00
457	A10	1493222.000	5.241	5.241	0.000	0.00
458	B10	3858229.000	16.293	16.293	0.000	0.00
459	C10	1712621.000	6.266	6.266	0.000	0.00
46	F6	561199.000	0.886	0.886	0.000	0.00
460	D10	1967021.000	7.455	7.455	0.000	0.00
461	E10	1059242.000	3.213	3.213	0.000	0.00
462	F10	1838602.000	6.855	6.855	0.000	0.00
463	G10	1703822.000	6.225	6.225	0.000	0.00
464	H10	544118.000	0.806	0.806	0.000	0.00
465	A11	605824.000	1.094	1.094	0.000	0.00
466	B11	1226301.000	3.994	3.994	0.000	0.00
467	C11	1779093.000	6.577	6.577	0.000	0.00
468	D11	1029805.000	3.075	3.075	0.000	0.00
469	E11	5169713.000	22.421	22.421	0.000	0.00
47	G6	2584846.000	10.342	10.342	0.000	0.00
470	F11	1272684.000	4.210	4.210	0.000	0.00
471	G11	3435988.000	14.320	14.320	0.000	0.00
472	H11	1062435.000	3.228	3.228	0.000	0.00

Unknowns_NoDiIn (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
48	H6	1080647.000	3.313	3.313	0.000	0.0
481	A1	3274002.000	13.563	13.563	0.000	0.0
482	B1	1490194.000	5.227	5.227	0.000	0.0
483	C1	6459502.000	28.448	28.448	0.000	0.0
484	D1	1452063.000	5.049	5.049	0.000	0.0
485	E1	1453712.000	5.056	5.056	0.000	0.0
486	F1	6949441.000	30.738	30.738	0.000	0.0
487	G1	7982653.000	35.566	35.566	0.000	0.0
488	H1	5863479.000	25.663	25.663	0.000	0.0
489	A2	6236499.000	27.406	27.406	0.000	0.0
49	A7	3282856.000	13.604	13.604	0.000	0.0
490	B2	2759071.000	11.156	11.156	0.000	0.0
491	C2	9430578.000	42.332	42.332	0.000	0.0
492	D2	4211965.000	17.946	17.946	0.000	0.0
493	E2	1225108.000	3.988	3.988	0.000	0.0
494	F2	6147954.000	26.992	26.992	0.000	0.0
495	G2	1454455.000	5.060	5.060	0.000	0.0
496	H2	580969.000	0.978	0.978	0.000	0.0
497	A3	6662385.000	29.396	29.396	0.000	0.0
498	B3	4122885.000	17.529	17.529	0.000	0.0
499	C3	2023260.000	7.718	7.718	0.000	0.0
50	B7	1431040.000	4.950	4.950	0.000	0.0
500	D3	2322567.000	9.117	9.117	0.000	0.0
501	E3	2072863.000	7.950	7.950	0.000	0.0
502	F3	2929350.000	11.952	11.952	0.000	0.0
503	G3	1631884.000	5.889	5.889	0.000	0.0
504	H3	6376417.000	28.060	28.060	0.000	0.0
505	A4	10994597.000	49.641	49.641	0.000	0.0
506	B4	3635347.000	15.251	15.251	0.000	0.0
507	C4	8323190.000	37.157	37.157	0.000	0.0
508	D4	1814125.000	6.741	6.741	0.000	0.0
509	E4	1126304.000	3.526	3.526	0.000	0.0
51	C7	2058519.000	7.883	7.883	0.000	0.0
510	F4	1534431.000	5.434	5.434	0.000	0.0
511	G4	6675250.000	29.457	29.457	0.000	0.0
512	H4	1308183.000	4.376	4.376	0.000	0.0
513	A5	2529792.000	10.085	10.085	0.000	0.0
514	B5	2075835.000	7.964	7.964	0.000	0.0
515	C5	74755.000	-1.387	-1.387	0.000	0.0
516	D5	743797.000	1.739	1.739	0.000	0.0
517	E5	1185023.000	3.801	3.801	0.000	0.0
518	F5	1053154.000	3.185	3.185	0.000	0.0
519	G5	570828.000	0.931	0.931	0.000	0.0
52	D7	74935.000	-1.387	-1.387	0.000	0.0
520	H5	72693.000	-1.397	-1.397	0.000	0.0
521	A6	1091108.000	3.362	3.362	0.000	0.0
522	B6	1548664.000	5.500	5.500	0.000	0.0
523	C6	1402551.000	4.817	4.817	0.000	0.0
524	D6	1251493.000	4.111	4.111	0.000	0.0
525	E6	2059520.000	7.887	7.887	0.000	0.0
526	F6	478294.000	0.498	0.498	0.000	0.0
527	G6	2351082.000	9.250	9.250	0.000	0.0
528	H6	1063050.000	3.231	3.231	0.000	0.0
529	A7	3054179.000	12.535	12.535	0.000	0.0
53	E7	1714676.000	6.276	6.276	0.000	0.0
530	B7	1304164.000	4.358	4.358	0.000	0.0
531	C7	1774185.000	6.554	6.554	0.000	0.0

Unknowns_NoDiIn (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
532	D7	114706.000	-1.201	-1.201	0.000	0.00
533	E7	1485079.000	5.203	5.203	0.000	0.00
534	F7	392633.000	0.098	0.098	0.000	0.00
535	G7	1350658.000	4.575	4.575	0.000	0.00
536	H7	3693897.000	15.525	15.525	0.000	0.00
537	A8	3514833.000	14.688	14.688	0.000	0.00
538	B8	1611772.000	5.795	5.795	0.000	0.00
539	C8	1840441.000	6.864	6.864	0.000	0.00
54	F7	419369.000	0.223	0.223	0.000	0.00
540	D8	791508.000	1.962	1.962	0.000	0.00
541	E8	3980561.000	16.864	16.864	0.000	0.00
542	F8	483319.000	0.522	0.522	0.000	0.00
543	G8	778433.000	1.901	1.901	0.000	0.00
544	H8	373503.000	0.009	0.009	0.000	0.00
545	A9	1006458.000	2.966	2.966	0.000	0.00
546	B9	2550285.000	10.181	10.181	0.000	0.00
547	C9	496755.000	0.585	0.585	0.000	0.00
548	D9	3663991.000	15.385	15.385	0.000	0.00
549	E9	3763361.000	15.849	15.849	0.000	0.00
55	G7	1511919.000	5.328	5.328	0.000	0.00
550	F9	4047252.000	17.176	17.176	0.000	0.00
551	G9	338374.000	-0.156	-0.156	0.000	0.00
552	H9	1551762.000	5.515	5.515	0.000	0.00
553	A10	1546907.000	5.492	5.492	0.000	0.00
554	B10	4145117.000	17.633	17.633	0.000	0.00
555	C10	1708036.000	6.245	6.245	0.000	0.00
556	D10	1991390.000	7.569	7.569	0.000	0.00
557	E10	1057464.000	3.205	3.205	0.000	0.00
558	F10	1868322.000	6.994	6.994	0.000	0.00
559	G10	1783331.000	6.597	6.597	0.000	0.00
56	H7	4048300.000	17.181	17.181	0.000	0.00
560	H10	559984.000	0.880	0.880	0.000	0.00
561	A11	651372.000	1.307	1.307	0.000	0.00
562	B11	1295792.000	4.318	4.318	0.000	0.00
563	C11	1780167.000	6.582	6.582	0.000	0.00
564	D11	549640.000	0.832	0.832	0.000	0.00
565	E11	5749932.000	25.133	25.133	0.000	0.00
566	F11	1273405.000	4.214	4.214	0.000	0.00
567	G11	3732986.000	15.707	15.707	0.000	0.00
568	H11	1052465.000	3.181	3.181	0.000	0.00
57	A8	3848668.000	16.248	16.248	0.000	0.00
577	A4	2532876.000	10.099	10.099	0.000	0.00
578	B4	1318990.000	4.427	4.427	0.000	0.00
579	C4	2625275.000	10.531	10.531	0.000	0.00
58	B8	1814375.000	6.742	6.742	0.000	0.00
580	D4	2190017.000	8.497	8.497	0.000	0.00
581	E4	805596.000	2.028	2.028	0.000	0.00
582	F4	1886323.000	7.078	7.078	0.000	0.00
583	G4	483734.000	0.524	0.524	0.000	0.00
584	H4	766579.000	1.845	1.845	0.000	0.00
585	A5	2561219.000	10.232	10.232	0.000	0.00
586	B5	1611495.000	5.794	5.794	0.000	0.00
587	C5	2856045.000	11.609	11.609	0.000	0.00
588	D5	2589577.000	10.364	10.364	0.000	0.00
589	E5	957431.000	2.737	2.737	0.000	0.00
59	C8	2596319.000	10.396	10.396	0.000	0.00
590	F5	2359322.000	9.288	9.288	0.000	0.00

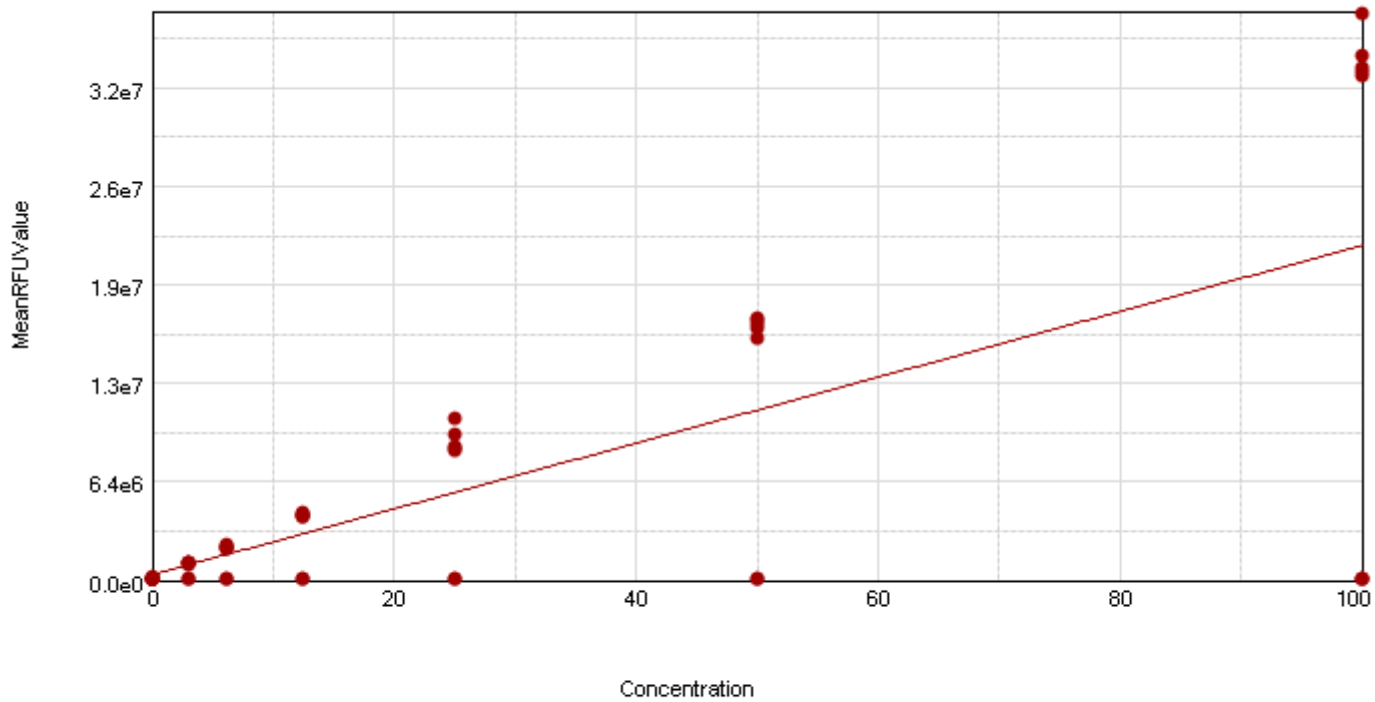
Unknowns_NoDiIn (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
591	G5	822783.000	2.108	2.108	0.000	0.0
592	H5	695565.000	1.514	1.514	0.000	0.0
593	A6	2866980.000	11.661	11.661	0.000	0.0
594	B6	1682598.000	6.126	6.126	0.000	0.0
595	C6	2870824.000	11.679	11.679	0.000	0.0
596	D6	2681080.000	10.792	10.792	0.000	0.0
597	E6	1020144.000	3.030	3.030	0.000	0.0
598	F6	2267050.000	8.857	8.857	0.000	0.0
599	G6	633385.000	1.223	1.223	0.000	0.0
60	D8	906565.000	2.500	2.500	0.000	0.0
600	H6	736648.000	1.706	1.706	0.000	0.0
601	A7	525419.000	0.718	0.718	0.000	0.0
602	B7	1709760.000	6.253	6.253	0.000	0.0
603	C7	3069339.000	12.606	12.606	0.000	0.0
604	D7	2656243.000	10.676	10.676	0.000	0.0
605	E7	1052906.000	3.183	3.183	0.000	0.0
606	F7	2530026.000	10.086	10.086	0.000	0.0
607	G7	559347.000	0.877	0.877	0.000	0.0
608	H7	645710.000	1.281	1.281	0.000	0.0
609	A8	548604.000	0.827	0.827	0.000	0.0
61	E8	4356625.000	18.622	18.622	0.000	0.0
610	B8	1750467.000	6.443	6.443	0.000	0.0
611	C8	3061536.000	12.570	12.570	0.000	0.0
612	D8	2721728.000	10.982	10.982	0.000	0.0
613	E8	1024797.000	3.052	3.052	0.000	0.0
614	F8	2525602.000	10.065	10.065	0.000	0.0
615	G8	580457.000	0.976	0.976	0.000	0.0
616	H8	655426.000	1.326	1.326	0.000	0.0
617	A9	506864.000	0.632	0.632	0.000	0.0
618	B9	1537241.000	5.447	5.447	0.000	0.0
619	C9	2809991.000	11.394	11.394	0.000	0.0
62	F8	612812.000	1.127	1.127	0.000	0.0
620	D9	2454986.000	9.735	9.735	0.000	0.0
621	E9	1048673.000	3.164	3.164	0.000	0.0
622	F9	2265170.000	8.848	8.848	0.000	0.0
623	G9	538355.000	0.779	0.779	0.000	0.0
624	H9	617247.000	1.148	1.148	0.000	0.0
63	G8	830453.000	2.144	2.144	0.000	0.0
64	H8	410881.000	0.183	0.183	0.000	0.0
65	A9	1270538.000	4.200	4.200	0.000	0.0
66	B9	2761313.000	11.167	11.167	0.000	0.0
67	C9	624814.000	1.183	1.183	0.000	0.0
68	D9	3813601.000	16.084	16.084	0.000	0.0
69	E9	4089856.000	17.375	17.375	0.000	0.0
70	F9	4440936.000	19.016	19.016	0.000	0.0
71	G9	373092.000	0.007	0.007	0.000	0.0
72	H9	1679985.000	6.114	6.114	0.000	0.0
73	A10	1724886.000	6.324	6.324	0.000	0.0
74	B10	4695587.000	20.206	20.206	0.000	0.0
75	C10	1866227.000	6.984	6.984	0.000	0.0
76	D10	2249118.000	8.773	8.773	0.000	0.0
77	E10	1102649.000	3.416	3.416	0.000	0.0
78	F10	2137682.000	8.253	8.253	0.000	0.0
79	G10	1988275.000	7.554	7.554	0.000	0.0
80	H10	655960.000	1.328	1.328	0.000	0.0
81	A11	721557.000	1.635	1.635	0.000	0.0
82	B11	1451104.000	5.044	5.044	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
83	C11	2149634.000	8.308	8.308	0.000	0.0
84	D11	653214.000	1.316	1.316	0.000	0.0
85	E11	6249419.000	27.467	27.467	0.000	0.0
86	F11	1334790.000	4.501	4.501	0.000	0.0
87	G11	4414546.000	18.892	18.892	0.000	0.0
88	H11	1222082.000	3.974	3.974	0.000	0.0

Standard Curve



● STD#1 (Standards: MeanRF... vs Concentr...)

Curve Fit Results ▼

Intro

Quantitation of double-stranded DNA using Quant-iT PicoGreen Reagent

Invitrogen (Molecular Probes)

MATERIALS

- o Quant-iT PicoGreen dsDNA Assay Kit, including lambda DNA standard (Invitrogen cat. #P7589 or P11496)
- o Black 96-well plate (Greiner Bio-One, cat. # 655096)
- o Brown or amber (light-blocking) microcentrifuge tubes

METHODS*Set up the protocol:*

- o Select Wells to Read and Assay Plate Type by clicking on "Settings" and locating the options on the left side of the screen.
- o Click the Template button to open a window where you can assign wells of the microplate to pre-set template groups using the drop-down menu to select the appropriate template group. There are preconfigured template groups in the PicoGreen Fluorescence protocol including Standards, Unknowns, and Unknowns_NoDiln (for undiluted samples). Assigning wells to pre-set template groups populates group tables in the protocol with the corresponding data acquired when the microplate is read.

Prepare the assay

The method for this assay follows the instructions in the product information sheet for Quant-iT PicoGreen dsDNA Reagent and Kits from Molecular Probes, except that the assay volume is proportionately reduced from 2.0 mL to 200 μ L to fit a 96-well microplate format.

- o Prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) by diluting the concentrated buffer from the kit 20-fold with distilled DNase-free water, as required by Molecular Probes.
- o Prepare an aqueous working solution of Quant-iT PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE buffer (prepared above). Preparation of the solution in a plastic container, rather than glass, is recommended, as the reagent may adsorb to glass surfaces. Protect the solution from light by using amber or brown tubes, or by covering with foil. This solution should be used within a few hours of its preparation.
- o DNA standard curve: Prepare a 2 μ g/mL stock solution of dsDNA in TE. The lambda DNA standard provided with the kit can be diluted 50-fold in TE to make the 2 μ g/mL solution. Note: in some cases it may be preferable to make the standard curve using DNA similar to the type being assayed.
- o A high-range standard curve may be prepared from 1 ng/mL to 1 μ g/mL, or a low-range standard curve may be prepared from 25 μ g/mL to 25 ng/mL. For the high-range curve, follow the dilution scheme shown in the PicoGreen product insert; for the low-range curve, dilute the 2 μ g/mL solution 40-fold to yield a 50 ng/mL solution, and refer to the alternative dilution scheme in the product insert.
- o Pipet standards into a solid black 96-well microplate at 100 μ L per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).
- o Add 100 μ L of the aqueous working solution of Quant-iT PicoGreen reagent to each well. Mix well by trituration or plate shaker and incubate for 2 to 5 minutes at room temperature, protected from light.

Read the microplate

- " Make sure the purple plate adapter is in the microplate reader drawer. Place the microplate in the drawer.
- " Click the Read button in the SoftMax Pro software. The instrument will read the plate and the relative fluorescence units will be displayed in the Plate section of the protocol.

Analyze the data

- o After the microplate has been read, the relative fluorescence units (RFUs) will be displayed in the Plate section. The data will be analyzed in the Group Tables that were created when the template was set up.
- o Standards assigned in the Template (and thus displayed in the Standards group table) will be automatically plotted in the Standard Curve section of the protocol. A linear curve fit is applied by default, but a log-log fit may be used when plotting a standard curve over a wide dynamic range. Curve fits are chosen from the drop-down Curve Fit menu in the graph section's tool bar.

READER SUITABILITY:

All SpectraMax readers with fluorescence capability.

PROTOCOL REVISION HISTORY:

- v 1.1: Imported from SMP 5.4.2 April 2011 (CLO & ELM)
- v 1.2: Emission wavelength changed from 540 nm to 525 nm. (CLO)

Plate01

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.5e7	3.9e7	3.7e7	2.5e6	2.5e6	2.8e6	5.1e5	5.3e5	4.9e5	6.3e4	6.2e4	6.2e4
B	1.5e7	1.6e7	1.6e7	1.3e6	1.5e6	1.6e6	1.6e6	1.7e6	1.4e6	6.6e4	6.3e4	6.2e4
C	7.8e6	8.1e6	8.0e6	2.5e6	2.8e6	2.8e6	3.0e6	2.9e6	2.7e6	6.4e4	6.3e4	1.3e5
D	3.8e6	3.9e6	3.9e6	2.1e6	2.5e6	2.6e6	2.5e6	2.6e6	2.3e6	6.6e4	6.3e4	6.4e4
E	1.8e6	1.8e6	2.0e6	7.7e5	9.2e5	9.8e5	1.0e6	9.8e5	1.0e6	6.5e4	6.4e4	6.5e4
F	8.9e5	9.9e5	9.7e5	1.8e6	2.2e6	2.2e6	2.4e6	2.4e6	2.2e6	6.2e4	6.3e4	6.7e4
G	6.2e4	6.2e4	6.3e4	4.7e5	9.9e5	6.1e5	5.4e5	5.6e5	5.2e5	7.9e4	6.3e4	6.2e4
H	6.1e4	6.8e4	6.2e4	7.0e5	6.6e5	6.9e5	6.1e5	6.2e5	5.8e5	6.3e4	6.1e4	6.2e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint
 Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:15 PM
 3/17/2014

Mean Temperature : 29.5 °C

Plate04

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.4e6	5.9e6	6.5e6	1.0e7	2.5e6	1.2e6	2.9e6	3.3e6	1.1e6	1.6e6	6.4e5	3.2e7
B	1.5e6	2.8e6	4.0e6	3.5e6	1.7e6	1.7e6	1.3e6	1.6e6	2.5e6	4.1e6	1.3e6	1.5e7
C	6.2e6	9.7e6	2.1e6	8.6e6	7.4e4	1.4e6	1.8e6	2.4e6	5.5e5	1.7e6	1.9e6	9.8e6
D	1.5e6	5.2e6	2.2e6	1.8e6	8.2e5	1.3e6	7.2e4	8.3e5	3.5e6	2.1e6	5.8e5	4.0e6
E	1.4e6	1.2e6	2.2e6	1.2e6	1.4e6	2.2e6	1.5e6	4.0e6	3.8e6	1.0e6	5.6e6	1.9e6
F	6.6e6	6.2e6	3.1e6	1.8e6	1.0e6	5.1e5	3.8e5	5.6e5	4.0e6	1.9e6	1.2e6	1.0e6
G	8.4e6	1.5e6	1.5e6	6.5e6	6.4e5	2.4e6	1.4e6	7.6e5	3.6e5	1.8e6	4.0e6	6.7e4
H	5.9e6	5.5e5	7.3e6	1.3e6	7.1e4	1.1e6	3.6e6	3.6e5	1.5e6	5.9e5	1.1e6	6.6e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint
 Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:17 PM
 3/17/2014

Mean Temperature : 29.5 °C

Plate05

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.0e6	5.7e6	6.1e6	1.1e7	2.5e6	1.1e6	2.8e6	3.2e6	8.5e5	1.6e6	6.6e5	3.3e7
B	1.4e6	2.6e6	3.8e6	3.4e6	1.9e6	1.9e6	1.2e6	1.5e6	2.3e6	4.3e6	1.2e6	1.6e7
C	6.5e6	8.9e6	1.9e6	8.2e6	7.4e4	1.3e6	1.7e6	1.9e6	4.9e5	1.7e6	1.8e6	8.0e6
D	1.4e6	4.2e6	2.4e6	1.7e6	7.3e5	1.2e6	7.3e4	7.5e5	3.4e6	2.0e6	5.1e5	4.0e6
E	1.4e6	1.2e6	1.9e6	1.1e6	1.2e6	2.1e6	1.5e6	3.8e6	3.9e6	1.0e6	5.8e6	2.1e6
F	7.3e6	6.2e6	3.0e6	1.6e6	9.9e5	4.6e5	3.9e5	4.9e5	3.9e6	1.9e6	1.3e6	1.1e6
G	7.9e6	1.5e6	1.7e6	6.4e6	5.3e5	2.4e6	1.3e6	7.7e5	3.4e5	1.6e6	3.9e6	6.8e4
H	5.9e6	5.8e5	6.9e6	1.3e6	7.2e4	9.9e5	3.9e6	3.8e5	1.4e6	4.9e5	1.0e6	6.8e4

Reduction Settings

Wavelength Combination : !Lm1

Settings Information

Endpoint

- Fluorescence
- Lm1 485, 535
- Slide(s) Ex1, Em1
- More Settings
- Shake Off
- ReadOrder Row
- Show Optimizer On
- PMT and Optics
- Integration Time 400 ms
- Read from Top
- Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:20 PM
 3/17/2014

Mean Temperature : 29.5 °C

Standards

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
01	100.000	132.418 149.615 140.466	A1 A2 A3	34858624.0... 39294788.0... 36934868.0...	37029426.667	22...	6.0
02	50.000	57.005 58.522 59.335	B1 B2 B3	15404886.0... 15796349.0... 16006026.0...	15735753.667	30...	1.9
03	25.000	27.499 28.634 28.411	C1 C2 C3	7793379.000 8086141.000 8028569.000	7969363.000	15...	1.9
04	12.500	12.154 12.330 12.322	D1 D2 D3	3834978.000 3880307.000 3878224.000	3864503.000	25...	0.7
05	6.250	4.237 4.430 4.955	E1 E2 E3	1792563.000 1842517.000 1978025.000	1871035.000	95...	5.1
06	3.125	0.731 1.126 1.061	F1 F2 F3	888359.000 990258.000 973335.000	950650.667	54...	5.7
07	0.000	-2.473 -2.471 -2.469	G1 G2 G3	61832.000 62273.000 62772.000	62292.333	47...	0.8
08	100.000	-2.477 -2.450 -2.470	H1 H2 H3	60837.000 67703.000 62424.000	63654.667	35...	5.6
09	0.000	-2.468 -2.471 -2.474 -2.457 -2.467 -2.472 -2.465 -2.468 -2.206 -2.457 -2.469 -2.466 -2.461 -2.466 -2.461 -2.470 -2.468 -2.453 -2.408 -2.467 -2.472 -2.470 -2.476 -2.472	A10 A11 A12 B10 B11 B12 C10 C11 C12 D10 D11 D12 E10 E11 E12 F10 F11 F12 G10 G11 G12 H10 H11 H12	62979.000 62139.000 61557.000 65809.000 63224.000 61915.000 63781.000 63003.000 130622.000 65819.000 62787.000 63599.000 64793.000 63660.000 64767.000 62457.000 63098.000 67028.000 78547.000 63183.000 62015.000 62626.000 60870.000 61976.000	66760.583	14...	21.0
26	100.000	123.133	A12	32463418.0...	32463418.000	0....	0.0
27	50.000	55.208	B12	14941452.0...	14941452.000	0....	0.0
28	25.000	35.235	C12	9788990.000	9788990.000	0....	0.0
29	12.500	12.768	D12	3993406.000	3993406.000	0....	0.0
30	6.250	4.844	E12	1949208.000	1949208.000	0....	0.0
31	3.125	1.319	F12	1039853.000	1039853.000	0....	0.0
32	0.000	-2.451	G12	67439.000	67439.000	0....	0.0
33	0.000	-2.456	H12	66051.000	66051.000	0....	0.0
34	100.000	123.732	A12	32617910.0...	32617910.000	0....	0.0
35	50.000	58.492	B12	15788590.0...	15788590.000	0....	0.0
36	25.000	28.113	C12	7951708.000	7951708.000	0....	0.0

Standards (Contd)

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
37	12.500	12.917	D12	4031802.000	4031802.000	0....	0.0
38	6.250	5.580	E12	2139070.000	2139070.000	0....	0.0
39	3.125	1.399	F12	1060684.000	1060684.000	0....	0.0
40	0.000	-2.450	G12	67724.000	67724.000	0....	0.0
41	0.000	-2.450	H12	67697.000	67697.000	0....	0.0

Unknowns

Sample	Wells	RFU_Values	Concentration	Mean_Conc	SD	CV	Dilution	AdjConc
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Unknowns_NoDiln

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
193	A1	3369141.000	10.348	10.348	0.000	0.0
194	B1	1503726.000	3.117	3.117	0.000	0.0
195	C1	6221018.000	21.404	21.404	0.000	0.0
196	D1	1516819.000	3.168	3.168	0.000	0.0
197	E1	1411027.000	2.757	2.757	0.000	0.0
198	F1	6624517.000	22.968	22.968	0.000	0.0
199	G1	8362911.000	29.707	29.707	0.000	0.0
200	H1	5949285.000	20.350	20.350	0.000	0.0
201	A2	5908607.000	20.192	20.192	0.000	0.0
202	B2	2760838.000	7.990	7.990	0.000	0.0
203	C2	9701343.000	34.895	34.895	0.000	0.0
204	D2	5236815.000	17.588	17.588	0.000	0.0
205	E2	1207010.000	1.967	1.967	0.000	0.0
206	F2	6223101.000	21.412	21.412	0.000	0.0
207	G2	1483518.000	3.039	3.039	0.000	0.0
208	H2	553121.000	-0.568	-0.568	0.000	0.0
209	A3	6494932.000	22.465	22.465	0.000	0.0
210	B3	3965939.000	12.662	12.662	0.000	0.0
211	C3	2105357.000	5.449	5.449	0.000	0.0
212	D3	2233804.000	5.947	5.947	0.000	0.0
213	E3	2159819.000	5.660	5.660	0.000	0.0
214	F3	3074353.000	9.205	9.205	0.000	0.0
215	G3	1507361.000	3.131	3.131	0.000	0.0
216	H3	7296274.000	25.572	25.572	0.000	0.0
217	A4	10261198.000	37.065	37.065	0.000	0.0
218	B4	3506205.000	10.879	10.879	0.000	0.0
219	C4	8587764.000	30.578	30.578	0.000	0.0
220	D4	1837764.000	4.412	4.412	0.000	0.0
221	E4	1150697.000	1.748	1.748	0.000	0.0
222	F4	1822946.000	4.354	4.354	0.000	0.0
223	G4	6531222.000	22.606	22.606	0.000	0.0
224	H4	1324609.000	2.422	2.422	0.000	0.0
225	A5	2463692.000	6.838	6.838	0.000	0.0
226	B5	1704813.000	3.896	3.896	0.000	0.0
227	C5	73558.000	-2.427	-2.427	0.000	0.0
228	D5	823305.000	0.479	0.479	0.000	0.0
229	E5	1353521.000	2.535	2.535	0.000	0.0
230	F5	1044659.000	1.337	1.337	0.000	0.0
231	G5	644971.000	-0.212	-0.212	0.000	0.0
232	H5	71045.000	-2.437	-2.437	0.000	0.0
233	A6	1165630.000	1.806	1.806	0.000	0.0
234	B6	1668407.000	3.755	3.755	0.000	0.0
235	C6	1399168.000	2.712	2.712	0.000	0.0
236	D6	1317347.000	2.394	2.394	0.000	0.0
237	E6	2150123.000	5.623	5.623	0.000	0.0
238	F6	505634.000	-0.752	-0.752	0.000	0.0
239	G6	2401615.000	6.598	6.598	0.000	0.0
240	H6	1050548.000	1.360	1.360	0.000	0.0
241	A7	2891399.000	8.496	8.496	0.000	0.0
242	B7	1297714.000	2.318	2.318	0.000	0.0
243	C7	1845634.000	4.442	4.442	0.000	0.0
244	D7	71555.000	-2.435	-2.435	0.000	0.0
245	E7	1544500.000	3.275	3.275	0.000	0.0
246	F7	375761.000	-1.256	-1.256	0.000	0.0
247	G7	1418851.000	2.788	2.788	0.000	0.0
248	H7	3627284.000	11.349	11.349	0.000	0.0
249	A8	3305840.000	10.103	10.103	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
250	B8	1563140.000	3.347	3.347	0.000	0.0
251	C8	2431894.000	6.715	6.715	0.000	0.0
252	D8	834553.000	0.523	0.523	0.000	0.0
253	E8	4031844.000	12.917	12.917	0.000	0.0
254	F8	560853.000	-0.538	-0.538	0.000	0.0
255	G8	761573.000	0.240	0.240	0.000	0.0
256	H8	360481.000	-1.315	-1.315	0.000	0.0
257	A9	1125384.000	1.650	1.650	0.000	0.0
258	B9	2530877.000	7.099	7.099	0.000	0.0
259	C9	554502.000	-0.563	-0.563	0.000	0.0
260	D9	3546001.000	11.034	11.034	0.000	0.0
261	E9	3766402.000	11.888	11.888	0.000	0.0
262	F9	3974864.000	12.696	12.696	0.000	0.0
263	G9	356106.000	-1.332	-1.332	0.000	0.0
264	H9	1465233.000	2.968	2.968	0.000	0.0
265	A10	1575779.000	3.396	3.396	0.000	0.0
266	B10	4107881.000	13.212	13.212	0.000	0.0
267	C10	1704134.000	3.894	3.894	0.000	0.0
268	D10	2081134.000	5.355	5.355	0.000	0.0
269	E10	1016765.000	1.229	1.229	0.000	0.0
270	F10	1862388.000	4.507	4.507	0.000	0.0
271	G10	1847097.000	4.448	4.448	0.000	0.0
272	H10	588328.000	-0.432	-0.432	0.000	0.0
273	A11	641646.000	-0.225	-0.225	0.000	0.0
274	B11	1324872.000	2.424	2.424	0.000	0.0
275	C11	1879006.000	4.572	4.572	0.000	0.0
276	D11	580341.000	-0.463	-0.463	0.000	0.0
277	E11	5616502.000	19.060	19.060	0.000	0.0
278	F11	1213379.000	1.991	1.991	0.000	0.0
279	G11	4022895.000	12.882	12.882	0.000	0.0
280	H11	1051325.000	1.363	1.363	0.000	0.0
289	A1	3015512.000	8.977	8.977	0.000	0.0
290	B1	1371188.000	2.603	2.603	0.000	0.0
291	C1	6533237.000	22.614	22.614	0.000	0.0
292	D1	1400500.000	2.717	2.717	0.000	0.0
293	E1	1407736.000	2.745	2.745	0.000	0.0
294	F1	7267251.000	25.459	25.459	0.000	0.0
295	G1	7886471.000	27.860	27.860	0.000	0.0
296	H1	5876567.000	20.068	20.068	0.000	0.0
297	A2	5654838.000	19.209	19.209	0.000	0.0
298	B2	2612645.000	7.416	7.416	0.000	0.0
299	C2	8882367.000	31.720	31.720	0.000	0.0
300	D2	4158635.000	13.409	13.409	0.000	0.0
301	E2	1215557.000	2.000	2.000	0.000	0.0
302	F2	6161887.000	21.174	21.174	0.000	0.0
303	G2	1456343.000	2.933	2.933	0.000	0.0
304	H2	584889.000	-0.445	-0.445	0.000	0.0
305	A3	6114851.000	20.992	20.992	0.000	0.0
306	B3	3813398.000	12.070	12.070	0.000	0.0
307	C3	1879187.000	4.572	4.572	0.000	0.0
308	D3	2424762.000	6.687	6.687	0.000	0.0
309	E3	1936534.000	4.795	4.795	0.000	0.0
310	F3	2969089.000	8.797	8.797	0.000	0.0
311	G3	1652519.000	3.694	3.694	0.000	0.0
312	H3	6901141.000	24.040	24.040	0.000	0.0
313	A4	11142135.000	40.480	40.480	0.000	0.0
314	B4	3384872.000	10.409	10.409	0.000	0.0

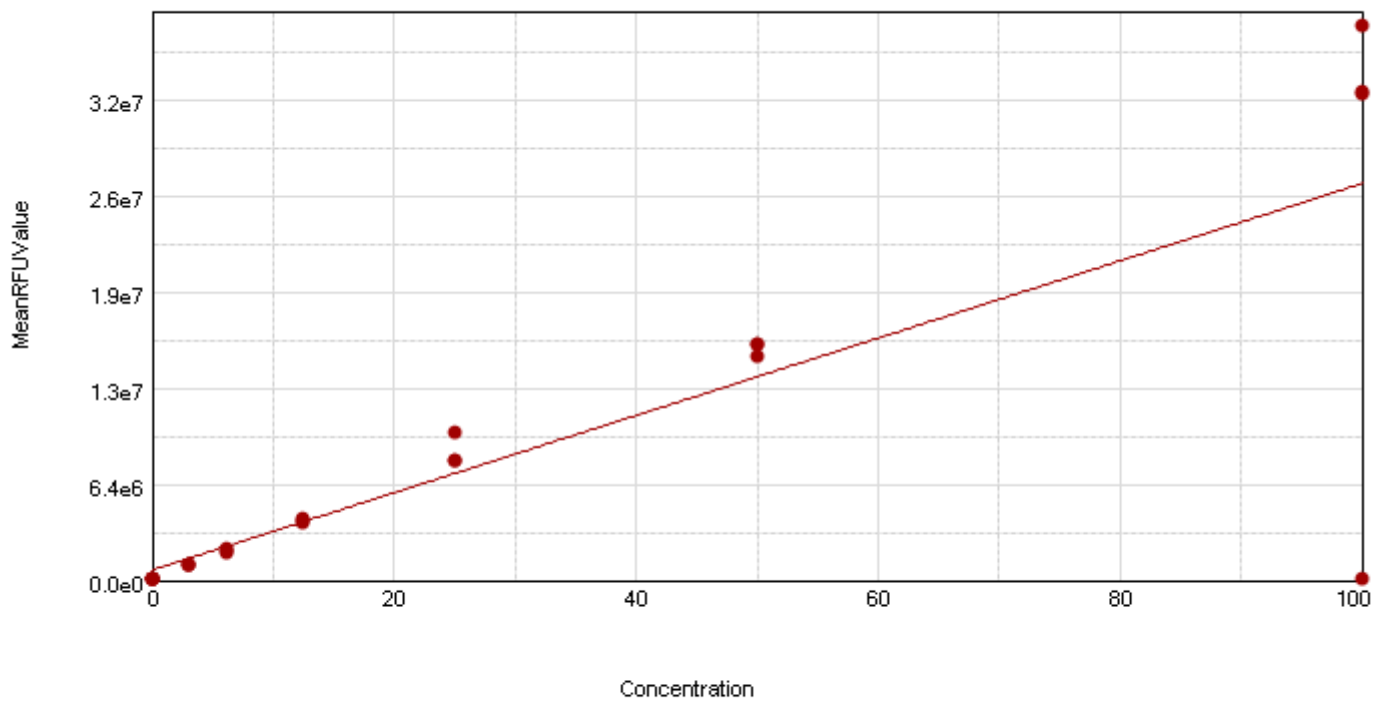
Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
315	C4	8187856.000	29.028	29.028	0.000	0.0
316	D4	1652445.000	3.693	3.693	0.000	0.0
317	E4	1066326.000	1.421	1.421	0.000	0.0
318	F4	1606915.000	3.517	3.517	0.000	0.0
319	G4	6402705.000	22.108	22.108	0.000	0.0
320	H4	1308695.000	2.361	2.361	0.000	0.0
321	A5	2476488.000	6.888	6.888	0.000	0.0
322	B5	1859635.000	4.497	4.497	0.000	0.0
323	C5	74055.000	-2.425	-2.425	0.000	0.0
324	D5	732800.000	0.128	0.128	0.000	0.0
325	E5	1174261.000	1.840	1.840	0.000	0.0
326	F5	988114.000	1.118	1.118	0.000	0.0
327	G5	527271.000	-0.668	-0.668	0.000	0.0
328	H5	71652.000	-2.435	-2.435	0.000	0.0
329	A6	1098814.000	1.547	1.547	0.000	0.0
330	B6	1874447.000	4.554	4.554	0.000	0.0
331	C6	1315607.000	2.388	2.388	0.000	0.0
332	D6	1223785.000	2.032	2.032	0.000	0.0
333	E6	2051622.000	5.241	5.241	0.000	0.0
334	F6	461237.000	-0.924	-0.924	0.000	0.0
335	G6	2395718.000	6.575	6.575	0.000	0.0
336	H6	986224.000	1.111	1.111	0.000	0.0
337	A7	2792225.000	8.112	8.112	0.000	0.0
338	B7	1227625.000	2.047	2.047	0.000	0.0
339	C7	1651436.000	3.689	3.689	0.000	0.0
340	D7	73234.000	-2.428	-2.428	0.000	0.0
341	E7	1467817.000	2.978	2.978	0.000	0.0
342	F7	394538.000	-1.183	-1.183	0.000	0.0
343	G7	1250216.000	2.134	2.134	0.000	0.0
344	H7	3864509.000	12.268	12.268	0.000	0.0
345	A8	3226682.000	9.796	9.796	0.000	0.0
346	B8	1543474.000	3.271	3.271	0.000	0.0
347	C8	1943752.000	4.823	4.823	0.000	0.0
348	D8	745352.000	0.177	0.177	0.000	0.0
349	E8	3837362.000	12.163	12.163	0.000	0.0
350	F8	485708.000	-0.830	-0.830	0.000	0.0
351	G8	774866.000	0.291	0.291	0.000	0.0
352	H8	381773.000	-1.232	-1.232	0.000	0.0
353	A9	851416.000	0.588	0.588	0.000	0.0
354	B9	2322920.000	6.292	6.292	0.000	0.0
355	C9	488473.000	-0.819	-0.819	0.000	0.0
356	D9	3445004.000	10.642	10.642	0.000	0.0
357	E9	3917884.000	12.475	12.475	0.000	0.0
358	F9	3920535.000	12.486	12.486	0.000	0.0
359	G9	339136.000	-1.398	-1.398	0.000	0.0
360	H9	1440790.000	2.873	2.873	0.000	0.0
361	A10	1606267.000	3.514	3.514	0.000	0.0
362	B10	4268955.000	13.836	13.836	0.000	0.0
363	C10	1690623.000	3.841	3.841	0.000	0.0
364	D10	1965316.000	4.906	4.906	0.000	0.0
365	E10	1039626.000	1.318	1.318	0.000	0.0
366	F10	1888865.000	4.610	4.610	0.000	0.0
367	G10	1629689.000	3.605	3.605	0.000	0.0
368	H10	489487.000	-0.815	-0.815	0.000	0.0
369	A11	664954.000	-0.135	-0.135	0.000	0.0
370	B11	1237830.000	2.086	2.086	0.000	0.0
371	C11	1800068.000	4.266	4.266	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
372	D11	514314.000	-0.719	-0.719	0.000	0.000
373	E11	5827384.000	19.878	19.878	0.000	0.000
374	F11	1322003.000	2.412	2.412	0.000	0.000
375	G11	3876712.000	12.316	12.316	0.000	0.000
376	H11	1028969.000	1.276	1.276	0.000	0.000
577	A4	2455806.000	6.808	6.808	0.000	0.000
578	B4	1251809.000	2.140	2.140	0.000	0.000
579	C4	2535063.000	7.115	7.115	0.000	0.000
580	D4	2106403.000	5.453	5.453	0.000	0.000
581	E4	772832.000	0.284	0.284	0.000	0.000
582	F4	1803347.000	4.278	4.278	0.000	0.000
583	G4	471363.000	-0.885	-0.885	0.000	0.000
584	H4	700064.000	0.001	0.001	0.000	0.000
585	A5	2473598.000	6.877	6.877	0.000	0.000
586	B5	1529027.000	3.215	3.215	0.000	0.000
587	C5	2760675.000	7.989	7.989	0.000	0.000
588	D5	2501788.000	6.986	6.986	0.000	0.000
589	E5	921432.000	0.860	0.860	0.000	0.000
590	F5	2248139.000	6.003	6.003	0.000	0.000
591	G5	990244.000	1.126	1.126	0.000	0.000
592	H5	657568.000	-0.163	-0.163	0.000	0.000
593	A6	2766881.000	8.013	8.013	0.000	0.000
594	B6	1588071.000	3.444	3.444	0.000	0.000
595	C6	2753464.000	7.961	7.961	0.000	0.000
596	D6	2580159.000	7.290	7.290	0.000	0.000
597	E6	977658.000	1.078	1.078	0.000	0.000
598	F6	2183916.000	5.754	5.754	0.000	0.000
599	G6	611342.000	-0.343	-0.343	0.000	0.000
600	H6	691860.000	-0.030	-0.030	0.000	0.000
601	A7	512405.000	-0.726	-0.726	0.000	0.000
602	B7	1618784.000	3.563	3.563	0.000	0.000
603	C7	2976976.000	8.828	8.828	0.000	0.000
604	D7	2545911.000	7.157	7.157	0.000	0.000
605	E7	1017301.000	1.231	1.231	0.000	0.000
606	F7	2433869.000	6.723	6.723	0.000	0.000
607	G7	542039.000	-0.611	-0.611	0.000	0.000
608	H7	611041.000	-0.344	-0.344	0.000	0.000
609	A8	534731.000	-0.639	-0.639	0.000	0.000
610	B8	1650411.000	3.685	3.685	0.000	0.000
611	C8	2935495.000	8.667	8.667	0.000	0.000
612	D8	2602009.000	7.374	7.374	0.000	0.000
613	E8	978949.000	1.083	1.083	0.000	0.000
614	F8	2405223.000	6.612	6.612	0.000	0.000
615	G8	559643.000	-0.543	-0.543	0.000	0.000
616	H8	622599.000	-0.299	-0.299	0.000	0.000
617	A9	489075.000	-0.816	-0.816	0.000	0.000
618	B9	1447593.000	2.899	2.899	0.000	0.000
619	C9	2694065.000	7.731	7.731	0.000	0.000
620	D9	2344897.000	6.378	6.378	0.000	0.000
621	E9	1002304.000	1.173	1.173	0.000	0.000
622	F9	2166396.000	5.686	5.686	0.000	0.000
623	G9	517793.000	-0.705	-0.705	0.000	0.000
624	H9	581493.000	-0.458	-0.458	0.000	0.000

Standard Curve



● STD#1 (Standards: MeanRF... vs Concentr...)

Curve Fit Results ▼

Intro

Quantitation of double-stranded DNA using Quant-iT PicoGreen Reagent

Invitrogen (Molecular Probes)

MATERIALS

- o Quant-iT PicoGreen dsDNA Assay Kit, including lambda DNA standard (Invitrogen cat. #P7589 or P11496)
- o Black 96-well plate (Greiner Bio-One, cat. # 655096)
- o Brown or amber (light-blocking) microcentrifuge tubes

METHODS*Set up the protocol:*

- o Select Wells to Read and Assay Plate Type by clicking on "Settings" and locating the options on the left side of the screen.
- o Click the Template button to open a window where you can assign wells of the microplate to pre-set template groups using the drop-down menu to select the appropriate template group. There are preconfigured template groups in the PicoGreen Fluorescence protocol including Standards, Unknowns, and Unknowns_NoDiln (for undiluted samples). Assigning wells to pre-set template groups populates group tables in the protocol with the corresponding data acquired when the microplate is read.

Prepare the assay

The method for this assay follows the instructions in the product information sheet for Quant-iT PicoGreen dsDNA Reagent and Kits from Molecular Probes, except that the assay volume is proportionately reduced from 2.0 mL to 200 μ L to fit a 96-well microplate format.

- o Prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) by diluting the concentrated buffer from the kit 20-fold with distilled DNase-free water, as required by Molecular Probes.
- o Prepare an aqueous working solution of Quant-iT PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE buffer (prepared above). Preparation of the solution in a plastic container, rather than glass, is recommended, as the reagent may adsorb to glass surfaces. Protect the solution from light by using amber or brown tubes, or by covering with foil. This solution should be used within a few hours of its preparation.
- o DNA standard curve: Prepare a 2 μ g/mL stock solution of dsDNA in TE. The lambda DNA standard provided with the kit can be diluted 50-fold in TE to make the 2 μ g/mL solution. Note: in some cases it may be preferable to make the standard curve using DNA similar to the type being assayed.
- o A high-range standard curve may be prepared from 1 ng/mL to 1 μ g/mL, or a low-range standard curve may be prepared from 25 μ g/mL to 25 ng/mL. For the high-range curve, follow the dilution scheme shown in the PicoGreen product insert; for the low-range curve, dilute the 2 μ g/mL solution 40-fold to yield a 50 ng/mL solution, and refer to the alternative dilution scheme in the product insert.
- o Pipet standards into a solid black 96-well microplate at 100 μ L per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).
- o Add 100 μ L of the aqueous working solution of Quant-iT PicoGreen reagent to each well. Mix well by trituration or plate shaker and incubate for 2 to 5 minutes at room temperature, protected from light.

Read the microplate

- " Make sure the purple plate adapter is in the microplate reader drawer. Place the microplate in the drawer.
- " Click the Read button in the SoftMax Pro software. The instrument will read the plate and the relative fluorescence units will be displayed in the Plate section of the protocol.

Analyze the data

- o After the microplate has been read, the relative fluorescence units (RFUs) will be displayed in the Plate section. The data will be analyzed in the Group Tables that were created when the template was set up.
- o Standards assigned in the Template (and thus displayed in the Standards group table) will be automatically plotted in the Standard Curve section of the protocol. A linear curve fit is applied by default, but a log-log fit may be used when plotting a standard curve over a wide dynamic range. Curve fits are chosen from the drop-down Curve Fit menu in the graph section's tool bar.

READER SUITABILITY:

All SpectraMax readers with fluorescence capability.

PROTOCOL REVISION HISTORY:

- v 1.1: Imported from SMP 5.4.2 April 2011 (CLO & ELM)
- v 1.2: Emission wavelength changed from 540 nm to 525 nm. (CLO)

Plate02

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.3e6	5.7e6	6.2e6	1.0e7	2.4e6	1.1e6	2.8e6	3.1e6	1.1e6	1.5e6	6.1e5	3.2e7
B	1.4e6	2.7e6	3.9e6	3.4e6	1.7e6	1.6e6	1.3e6	1.5e6	2.4e6	3.9e6	1.3e6	1.4e7
C	6.0e6	9.4e6	2.0e6	8.4e6	7.4e4	1.3e6	1.8e6	2.4e6	5.4e5	1.7e6	1.8e6	9.6e6
D	1.5e6	5.1e6	2.1e6	1.8e6	8.0e5	1.3e6	7.1e4	8.1e5	3.4e6	2.0e6	5.6e5	3.8e6
E	1.4e6	1.2e6	2.1e6	1.1e6	1.3e6	2.0e6	1.5e6	3.9e6	3.7e6	9.8e5	5.4e6	1.9e6
F	6.4e6	6.0e6	3.0e6	1.7e6	1.0e6	4.9e5	3.6e5	5.5e5	3.9e6	1.8e6	1.2e6	9.9e5
G	8.1e6	1.4e6	1.5e6	6.3e6	6.3e5	2.3e6	1.4e6	7.4e5	3.5e5	1.8e6	3.9e6	6.6e4
H	5.8e6	5.3e5	7.1e6	1.3e6	7.0e4	1.0e6	3.5e6	3.5e5	1.4e6	5.7e5	1.0e6	6.6e4

Settings Information

Endpoint
 Fluorescence
 Lm1 485,535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:29 PM
 3/17/2014

Mean Temperature : 29.5 °C

Reduction Settings

Wavelength Combination : !Lm1

Standards

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
1	100.000	101.290	A12	32349768.0...	32349768.000	0....	0.0
2	50.000	45.226	B12	14477659.0...	14477659.000	0....	0.0
3	25.000	29.896	C12	9590765.000	9590765.000	0....	0.0
4	12.500	11.805	D12	3823913.000	3823913.000	0....	0.0
5	6.250	5.715	E12	1882428.000	1882428.000	0....	0.0
6	3.125	2.926	F12	993420.000	993420.000	0....	0.0
7	0.000	0.018	G12	66399.000	66126.000	38...	0.6
		0.016	H12	65853.000			

Unknowns

Sample	Wells	RFU_Values	Concentration	Mean_Conc	SD	CV	Dilution	AdjConc
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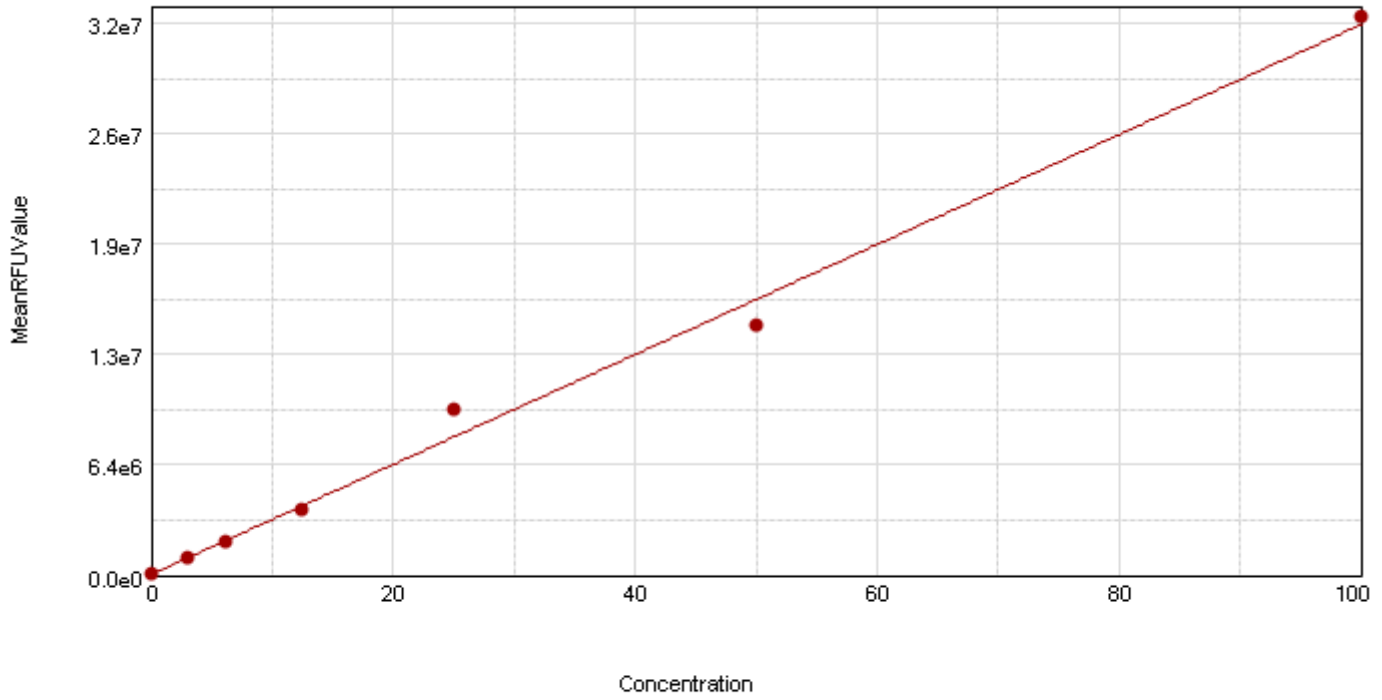
Unknowns_NoDiln

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
01	A1	3254939.000	10.021	10.021	0.000	0.0
02	B1	1443310.000	4.338	4.338	0.000	0.0
03	C1	5975569.000	18.555	18.555	0.000	0.0
04	D1	1451835.000	4.364	4.364	0.000	0.0
05	E1	1362276.000	4.083	4.083	0.000	0.0
06	F1	6367985.000	19.786	19.786	0.000	0.0
07	G1	8107476.000	25.243	25.243	0.000	0.0
08	H1	5808283.000	18.030	18.030	0.000	0.0
09	A2	5696717.000	17.680	17.680	0.000	0.0
10	B2	2681008.000	8.220	8.220	0.000	0.0
11	C2	9440488.000	29.424	29.424	0.000	0.0
12	D2	5056422.000	15.672	15.672	0.000	0.0
13	E2	1155936.000	3.436	3.436	0.000	0.0
14	F2	6044708.000	18.772	18.772	0.000	0.0
15	G2	1431919.000	4.302	4.302	0.000	0.0
16	H2	531841.000	1.478	1.478	0.000	0.0
17	A3	6214743.000	19.305	19.305	0.000	0.0
18	B3	3862127.000	11.925	11.925	0.000	0.0
19	C3	2028692.000	6.174	6.174	0.000	0.0
20	D3	2145809.000	6.541	6.541	0.000	0.0
21	E3	2096775.000	6.387	6.387	0.000	0.0
22	F3	2995188.000	9.206	9.206	0.000	0.0
23	G3	1471394.000	4.426	4.426	0.000	0.0
24	H3	7108359.000	22.109	22.109	0.000	0.0
25	A4	10208211.000	31.833	31.833	0.000	0.0
26	B4	3422947.000	10.548	10.548	0.000	0.0
27	C4	8372087.000	26.073	26.073	0.000	0.0
28	D4	1763514.000	5.342	5.342	0.000	0.0
29	E4	1101672.000	3.266	3.266	0.000	0.0
30	F4	1749946.000	5.299	5.299	0.000	0.0
31	G4	6341077.000	19.702	19.702	0.000	0.0
32	H4	1289732.000	3.856	3.856	0.000	0.0
33	A5	2373554.000	7.256	7.256	0.000	0.0
34	B5	1654978.000	5.001	5.001	0.000	0.0
35	C5	73873.000	0.042	0.042	0.000	0.0
36	D5	796235.000	2.308	2.308	0.000	0.0
37	E5	1311335.000	3.924	3.924	0.000	0.0
38	F5	1010648.000	2.980	2.980	0.000	0.0
39	G5	628075.000	1.780	1.780	0.000	0.0
40	H5	70351.000	0.031	0.031	0.000	0.0
41	A6	1127720.000	3.348	3.348	0.000	0.0
42	B6	1611909.000	4.866	4.866	0.000	0.0
43	C6	1341385.000	4.018	4.018	0.000	0.0
44	D6	1277225.000	3.816	3.816	0.000	0.0
45	E6	2036302.000	6.198	6.198	0.000	0.0
46	F6	490895.000	1.350	1.350	0.000	0.0
47	G6	2330766.000	7.121	7.121	0.000	0.0
48	H6	1015619.000	2.996	2.996	0.000	0.0
49	A7	2768518.000	8.495	8.495	0.000	0.0
50	B7	1251503.000	3.736	3.736	0.000	0.0
51	C7	1758053.000	5.325	5.325	0.000	0.0
52	D7	70969.000	0.033	0.033	0.000	0.0
53	E7	1483707.000	4.464	4.464	0.000	0.0
54	F7	364715.000	0.954	0.954	0.000	0.0
55	G7	1382413.000	4.146	4.146	0.000	0.0
56	H7	3510610.000	10.823	10.823	0.000	0.0
57	A8	3148662.000	9.687	9.687	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
58	B8	1505884.000	4.534	4.534	0.000	0.0
59	C8	2389060.000	7.304	7.304	0.000	0.0
60	D8	810771.000	2.353	2.353	0.000	0.0
61	E8	3938546.000	12.165	12.165	0.000	0.0
62	F8	546265.000	1.524	1.524	0.000	0.0
63	G8	742668.000	2.140	2.140	0.000	0.0
64	H8	350228.000	0.909	0.909	0.000	0.0
65	A9	1073989.000	3.179	3.179	0.000	0.0
66	B9	2413141.000	7.380	7.380	0.000	0.0
67	C9	535960.000	1.491	1.491	0.000	0.0
68	D9	3376159.000	10.401	10.401	0.000	0.0
69	E9	3658332.000	11.286	11.286	0.000	0.0
70	F9	3903383.000	12.055	12.055	0.000	0.0
71	G9	348279.000	0.902	0.902	0.000	0.0
72	H9	1421240.000	4.268	4.268	0.000	0.0
73	A10	1515934.000	4.565	4.565	0.000	0.0
74	B10	3922091.000	12.113	12.113	0.000	0.0
75	C10	1653879.000	4.998	4.998	0.000	0.0
76	D10	2022654.000	6.155	6.155	0.000	0.0
77	E10	983482.000	2.895	2.895	0.000	0.0
78	F10	1775795.000	5.380	5.380	0.000	0.0
79	G10	1813343.000	5.498	5.498	0.000	0.0
80	H10	566766.000	1.588	1.588	0.000	0.0
81	A11	614435.000	1.737	1.737	0.000	0.0
82	B11	1270638.000	3.796	3.796	0.000	0.0
83	C11	1820822.000	5.522	5.522	0.000	0.0
84	D11	560252.000	1.567	1.567	0.000	0.0
85	E11	5446355.000	16.895	16.895	0.000	0.0
86	F11	1178421.000	3.507	3.507	0.000	0.0
87	G11	3911197.000	12.079	12.079	0.000	0.0
88	H11	1017698.000	3.002	3.002	0.000	0.0

Standard Curve



● STD#1 (Standards: MeanRF... vs Concentr...)

Curve Fit Results ▼

Intro

Quantitation of double-stranded DNA using Quant-iT PicoGreen Reagent

Invitrogen (Molecular Probes)

MATERIALS

- o Quant-iT PicoGreen dsDNA Assay Kit, including lambda DNA standard (Invitrogen cat. #P7589 or P11496)
- o Black 96-well plate (Greiner Bio-One, cat. # 655096)
- o Brown or amber (light-blocking) microcentrifuge tubes

METHODS*Set up the protocol:*

- o Select Wells to Read and Assay Plate Type by clicking on "Settings" and locating the options on the left side of the screen.
- o Click the Template button to open a window where you can assign wells of the microplate to pre-set template groups using the drop-down menu to select the appropriate template group. There are preconfigured template groups in the PicoGreen Fluorescence protocol including Standards, Unknowns, and Unknowns_NoDiln (for undiluted samples). Assigning wells to pre-set template groups populates group tables in the protocol with the corresponding data acquired when the microplate is read.

Prepare the assay

The method for this assay follows the instructions in the product information sheet for Quant-iT PicoGreen dsDNA Reagent and Kits from Molecular Probes, except that the assay volume is proportionately reduced from 2.0 mL to 200 μ L to fit a 96-well microplate format.

- o Prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) by diluting the concentrated buffer from the kit 20-fold with distilled DNase-free water, as required by Molecular Probes.
- o Prepare an aqueous working solution of Quant-iT PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE buffer (prepared above). Preparation of the solution in a plastic container, rather than glass, is recommended, as the reagent may adsorb to glass surfaces. Protect the solution from light by using amber or brown tubes, or by covering with foil. This solution should be used within a few hours of its preparation.
- o DNA standard curve: Prepare a 2 μ g/mL stock solution of dsDNA in TE. The lambda DNA standard provided with the kit can be diluted 50-fold in TE to make the 2 μ g/mL solution. Note: in some cases it may be preferable to make the standard curve using DNA similar to the type being assayed.
- o A high-range standard curve may be prepared from 1 ng/mL to 1 μ g/mL, or a low-range standard curve may be prepared from 25 μ g/mL to 25 ng/mL. For the high-range curve, follow the dilution scheme shown in the PicoGreen product insert; for the low-range curve, dilute the 2 μ g/mL solution 40-fold to yield a 50 ng/mL solution, and refer to the alternative dilution scheme in the product insert.
- o Pipet standards into a solid black 96-well microplate at 100 μ L per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).
- o Add 100 μ L of the aqueous working solution of Quant-iT PicoGreen reagent to each well. Mix well by trituration or plate shaker and incubate for 2 to 5 minutes at room temperature, protected from light.

Read the microplate

- " Make sure the purple plate adapter is in the microplate reader drawer. Place the microplate in the drawer.
- " Click the Read button in the SoftMax Pro software. The instrument will read the plate and the relative fluorescence units will be displayed in the Plate section of the protocol.

Analyze the data

- o After the microplate has been read, the relative fluorescence units (RFUs) will be displayed in the Plate section. The data will be analyzed in the Group Tables that were created when the template was set up.
- o Standards assigned in the Template (and thus displayed in the Standards group table) will be automatically plotted in the Standard Curve section of the protocol. A linear curve fit is applied by default, but a log-log fit may be used when plotting a standard curve over a wide dynamic range. Curve fits are chosen from the drop-down Curve Fit menu in the graph section's tool bar.

READER SUITABILITY:

All SpectraMax readers with fluorescence capability.

PROTOCOL REVISION HISTORY:

- v 1.1: Imported from SMP 5.4.2 April 2011 (CLO & ELM)
- v 1.2: Emission wavelength changed from 540 nm to 525 nm. (CLO)

Plate02

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.9e6	5.5e6	5.9e6	1.1e7	2.4e6	1.1e6	2.7e6	3.1e6	8.2e5	1.5e6	6.3e5	3.3e7
B	1.3e6	2.5e6	3.7e6	3.3e6	1.8e6	1.9e6	1.2e6	1.5e6	2.2e6	4.1e6	1.2e6	1.5e7
C	6.4e6	8.7e6	1.8e6	8.0e6	7.3e4	1.3e6	1.6e6	1.9e6	4.7e5	1.7e6	1.8e6	7.8e6
D	1.4e6	4.0e6	2.3e6	1.6e6	7.2e5	1.2e6	7.2e4	7.3e5	3.3e6	1.9e6	5.0e5	3.9e6
E	1.4e6	1.2e6	1.9e6	1.0e6	1.1e6	2.0e6	1.4e6	3.7e6	3.8e6	1.0e6	5.7e6	2.0e6
F	7.0e6	5.9e6	2.9e6	1.5e6	9.6e5	4.5e5	3.9e5	4.8e5	3.8e6	1.8e6	1.3e6	1.0e6
G	7.7e6	1.4e6	1.6e6	6.2e6	5.2e5	2.3e6	1.2e6	7.6e5	3.4e5	1.6e6	3.8e6	6.7e4
H	5.6e6	5.6e5	6.7e6	1.3e6	7.1e4	9.5e5	3.8e6	3.7e5	1.4e6	4.8e5	1.0e6	6.8e4

Settings Information

Endpoint
 Fluorescence
 Lm1 485,535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:33 PM
 3/17/2014

Mean Temperature : 29.5 °C

Reduction Settings

Wavelength Combination : !Lm1

Standards

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
1	100.000	101.903	A12	33445150.0...	33445150.000	0....	0.0
2	50.000	46.376	B12	15086277.0...	15086277.000	0....	0.0
3	25.000	24.376	C12	7812252.000	7812252.000	0....	0.0
4	12.500	12.497	D12	3884586.000	3884586.000	0....	0.0
5	6.250	6.943	E12	2048177.000	2048177.000	0....	0.0
6	3.125	3.828	F12	1018214.000	1018214.000	0....	0.0
7	0.000	0.952	G12	67434.000	67595.500	22...	0.3
		0.953	H12	67757.000			

Unknowns

Sample	Wells	RFU_Values	Concentration	Mean_Conc	SD	CV	Dilution	AdjConc
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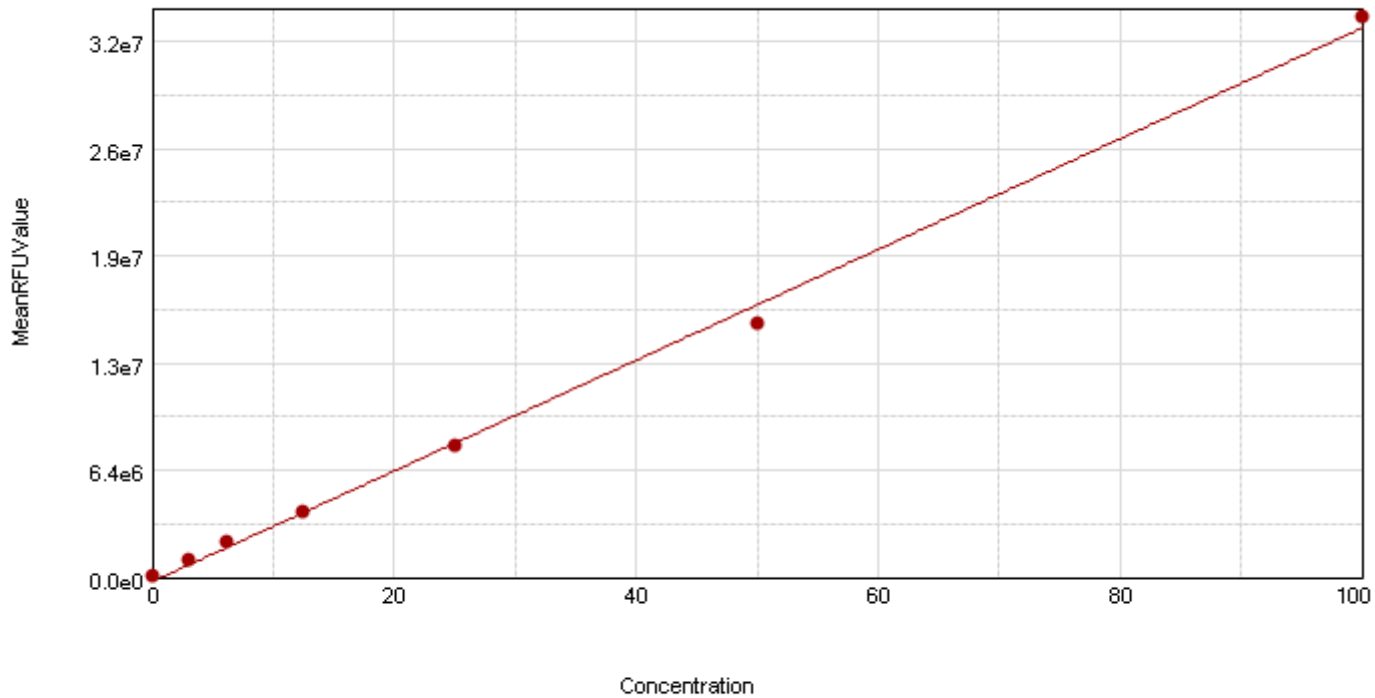
Unknowns_NoDiln

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
01	A1	2913692.000	9.560	9.560	0.000	0.0
02	B1	1335994.000	4.789	4.789	0.000	0.0
03	C1	6353897.000	19.965	19.965	0.000	0.0
04	D1	1358267.000	4.856	4.856	0.000	0.0
05	E1	1373995.000	4.904	4.904	0.000	0.0
06	F1	6995143.000	21.905	21.905	0.000	0.0
07	G1	7650106.000	23.886	23.886	0.000	0.0
08	H1	5646362.000	17.825	17.825	0.000	0.0
09	A2	5501995.000	17.389	17.389	0.000	0.0
10	B2	2536441.000	8.419	8.419	0.000	0.0
11	C2	8692080.000	27.037	27.037	0.000	0.0
12	D2	4025327.000	12.923	12.923	0.000	0.0
13	E2	1174108.000	4.299	4.299	0.000	0.0
14	F2	5927239.000	18.675	18.675	0.000	0.0
15	G2	1410196.000	5.013	5.013	0.000	0.0
16	H2	563908.000	2.454	2.454	0.000	0.0
17	A3	5906423.000	18.612	18.612	0.000	0.0
18	B3	3672681.000	11.856	11.856	0.000	0.0
19	C3	1807995.000	6.216	6.216	0.000	0.0
20	D3	2339066.000	7.822	7.822	0.000	0.0
21	E3	1875625.000	6.421	6.421	0.000	0.0
22	F3	2890086.000	9.489	9.489	0.000	0.0
23	G3	1597581.000	5.580	5.580	0.000	0.0
24	H3	6732709.000	21.111	21.111	0.000	0.0
25	A4	10896712.000	33.705	33.705	0.000	0.0
26	B4	3254046.000	10.590	10.590	0.000	0.0
27	C4	7987937.000	24.907	24.907	0.000	0.0
28	D4	1608292.000	5.612	5.612	0.000	0.0
29	E4	1035117.000	3.879	3.879	0.000	0.0
30	F4	1549108.000	5.433	5.433	0.000	0.0
31	G4	6238054.000	19.615	19.615	0.000	0.0
32	H4	1264640.000	4.573	4.573	0.000	0.0
33	A5	2393932.000	7.988	7.988	0.000	0.0
34	B5	1792013.000	6.168	6.168	0.000	0.0
35	C5	72898.000	0.968	0.968	0.000	0.0
36	D5	715327.000	2.911	2.911	0.000	0.0
37	E5	1140751.000	4.198	4.198	0.000	0.0
38	F5	956095.000	3.640	3.640	0.000	0.0
39	G5	516044.000	2.309	2.309	0.000	0.0
40	H5	71437.000	0.964	0.964	0.000	0.0
41	A6	1075593.000	4.001	4.001	0.000	0.0
42	B6	1857704.000	6.367	6.367	0.000	0.0
43	C6	1272898.000	4.598	4.598	0.000	0.0
44	D6	1198510.000	4.373	4.373	0.000	0.0
45	E6	1986237.000	6.755	6.755	0.000	0.0
46	F6	449664.000	2.108	2.108	0.000	0.0
47	G6	2332904.000	7.804	7.804	0.000	0.0
48	H6	951090.000	3.625	3.625	0.000	0.0
49	A7	2693216.000	8.894	8.894	0.000	0.0
50	B7	1184774.000	4.331	4.331	0.000	0.0
51	C7	1585985.000	5.545	5.545	0.000	0.0
52	D7	72237.000	0.966	0.966	0.000	0.0
53	E7	1402768.000	4.991	4.991	0.000	0.0
54	F7	385004.000	1.912	1.912	0.000	0.0
55	G7	1224455.000	4.451	4.451	0.000	0.0
56	H7	3775546.000	12.167	12.167	0.000	0.0
57	A8	3083285.000	10.073	10.073	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
58	B8	1486924.000	5.245	5.245	0.000	0.0
59	C8	1915740.000	6.542	6.542	0.000	0.0
60	D8	730485.000	2.957	2.957	0.000	0.0
61	E8	3735891.000	12.047	12.047	0.000	0.0
62	F8	477369.000	2.192	2.192	0.000	0.0
63	G8	760906.000	3.049	3.049	0.000	0.0
64	H8	368310.000	1.862	1.862	0.000	0.0
65	A9	821110.000	3.231	3.231	0.000	0.0
66	B9	2233764.000	7.504	7.504	0.000	0.0
67	C9	474960.000	2.184	2.184	0.000	0.0
68	D9	3306394.000	10.748	10.748	0.000	0.0
69	E9	3796094.000	12.229	12.229	0.000	0.0
70	F9	3821540.000	12.306	12.306	0.000	0.0
71	G9	337840.000	1.770	1.770	0.000	0.0
72	H9	1402552.000	4.990	4.990	0.000	0.0
73	A10	1546384.000	5.425	5.425	0.000	0.0
74	B10	4074552.000	13.071	13.071	0.000	0.0
75	C10	1651323.000	5.742	5.742	0.000	0.0
76	D10	1921292.000	6.559	6.559	0.000	0.0
77	E10	999744.000	3.772	3.772	0.000	0.0
78	F10	1811529.000	6.227	6.227	0.000	0.0
79	G10	1592832.000	5.565	5.565	0.000	0.0
80	H10	476205.000	2.188	2.188	0.000	0.0
81	A11	634735.000	2.668	2.668	0.000	0.0
82	B11	1192721.000	4.355	4.355	0.000	0.0
83	C11	1756662.000	6.061	6.061	0.000	0.0
84	D11	495112.000	2.245	2.245	0.000	0.0
85	E11	5657201.000	17.858	17.858	0.000	0.0
86	F11	1284867.000	4.634	4.634	0.000	0.0
87	G11	3753280.000	12.100	12.100	0.000	0.0
88	H11	997542.000	3.765	3.765	0.000	0.0

Standard Curve



● STD#1 (Standards: MeanRF... vs Concentr...)

Curve Fit Results ▼

Intro

Quantitation of double-stranded DNA using Quant-iT PicoGreen Reagent

Invitrogen (Molecular Probes)

MATERIALS

- o Quant-iT PicoGreen dsDNA Assay Kit, including lambda DNA standard (Invitrogen cat. #P7589 or P11496)
- o Black 96-well plate (Greiner Bio-One, cat. # 655096)
- o Brown or amber (light-blocking) microcentrifuge tubes

METHODS*Set up the protocol:*

- o Select Wells to Read and Assay Plate Type by clicking on "Settings" and locating the options on the left side of the screen.
- o Click the Template button to open a window where you can assign wells of the microplate to pre-set template groups using the drop-down menu to select the appropriate template group. There are preconfigured template groups in the PicoGreen Fluorescence protocol including Standards, Unknowns, and Unknowns_NoDiln (for undiluted samples). Assigning wells to pre-set template groups populates group tables in the protocol with the corresponding data acquired when the microplate is read.

Prepare the assay

The method for this assay follows the instructions in the product information sheet for Quant-iT PicoGreen dsDNA Reagent and Kits from Molecular Probes, except that the assay volume is proportionately reduced from 2.0 mL to 200 μ L to fit a 96-well microplate format.

- o Prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) by diluting the concentrated buffer from the kit 20-fold with distilled DNase-free water, as required by Molecular Probes.
- o Prepare an aqueous working solution of Quant-iT PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE buffer (prepared above). Preparation of the solution in a plastic container, rather than glass, is recommended, as the reagent may adsorb to glass surfaces. Protect the solution from light by using amber or brown tubes, or by covering with foil. This solution should be used within a few hours of its preparation.
- o DNA standard curve: Prepare a 2 μ g/mL stock solution of dsDNA in TE. The lambda DNA standard provided with the kit can be diluted 50-fold in TE to make the 2 μ g/mL solution. Note: in some cases it may be preferable to make the standard curve using DNA similar to the type being assayed.
- o A high-range standard curve may be prepared from 1 ng/mL to 1 μ g/mL, or a low-range standard curve may be prepared from 25 μ g/mL to 25 ng/mL. For the high-range curve, follow the dilution scheme shown in the PicoGreen product insert; for the low-range curve, dilute the 2 μ g/mL solution 40-fold to yield a 50 ng/mL solution, and refer to the alternative dilution scheme in the product insert.
- o Pipet standards into a solid black 96-well microplate at 100 μ L per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).
- o Add 100 μ L of the aqueous working solution of Quant-iT PicoGreen reagent to each well. Mix well by trituration or plate shaker and incubate for 2 to 5 minutes at room temperature, protected from light.

Read the microplate

- " Make sure the purple plate adapter is in the microplate reader drawer. Place the microplate in the drawer.
- " Click the Read button in the SoftMax Pro software. The instrument will read the plate and the relative fluorescence units will be displayed in the Plate section of the protocol.

Analyze the data

- o After the microplate has been read, the relative fluorescence units (RFUs) will be displayed in the Plate section. The data will be analyzed in the Group Tables that were created when the template was set up.
- o Standards assigned in the Template (and thus displayed in the Standards group table) will be automatically plotted in the Standard Curve section of the protocol. A linear curve fit is applied by default, but a log-log fit may be used when plotting a standard curve over a wide dynamic range. Curve fits are chosen from the drop-down Curve Fit menu in the graph section's tool bar.

READER SUITABILITY:

All SpectraMax readers with fluorescence capability.

PROTOCOL REVISION HISTORY:

- v 1.1: Imported from SMP 5.4.2 April 2011 (CLO & ELM)
- v 1.2: Emission wavelength changed from 540 nm to 525 nm. (CLO)

Plate02

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.9e6	5.4e6	5.6e6	9.9e6	2.3e6	9.8e5	2.8e6	3.1e6	9.0e5	1.4e6	5.7e5	3.4e7
B	1.3e6	2.5e6	3.5e6	3.3e6	1.9e6	1.4e6	1.2e6	1.4e6	2.3e6	3.5e6	1.2e6	1.6e7
C	5.7e6	8.3e6	1.8e6	7.2e6	7.3e4	1.3e6	1.6e6	1.7e6	4.6e5	1.6e6	1.6e6	7.8e6
D	1.3e6	3.9e6	2.1e6	1.6e6	6.7e5	1.2e6	6.9e4	7.2e5	3.3e6	1.9e6	8.1e5	3.9e6
E	1.3e6	1.1e6	1.8e6	9.7e5	1.1e6	1.9e6	1.4e6	3.6e6	3.5e6	1.0e6	4.9e6	2.0e6
F	6.5e6	5.9e6	2.6e6	1.5e6	9.7e5	4.5e5	3.7e5	4.7e5	3.6e6	1.7e6	1.2e6	1.0e6
G	7.0e6	1.3e6	1.5e6	6.1e6	5.3e5	2.2e6	1.2e6	7.4e5	2.9e5	1.6e6	3.2e6	6.6e4
H	5.3e6	5.4e5	6.0e6	1.2e6	6.9e4	9.6e5	3.5e6	4.0e5	1.5e6	5.1e5	1.0e6	6.5e4

Settings Information

Endpoint
 Fluorescence
 Lm1 485,535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:36 PM
 3/17/2014

Mean Temperature : 29.5 °C

Reduction Settings

Wavelength Combination : !Lm1

Standards

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
1	100.000	100.956	A12	33767712.0...	33767712.000	0....	0.0
2	50.000	48.635	B12	16144867.0...	16144867.000	0....	0.0
3	25.000	23.916	C12	7818875.000	7818875.000	0....	0.0
4	12.500	12.179	D12	3865575.000	3865575.000	0....	0.0
5	6.250	6.532	E12	1963660.000	1963660.000	0....	0.0
6	3.125	3.759	F12	1029754.000	1029754.000	0....	0.0
7	0.000	0.899	G12	66260.000	65777.000	68...	1.0
		0.896	H12	65294.000			

Unknowns

Sample	Wells	RFU_Values	Concentration	Mean_Conc	SD	CV	Dilution	AdjConc
--------	-------	------------	---------------	-----------	----	----	----------	---------

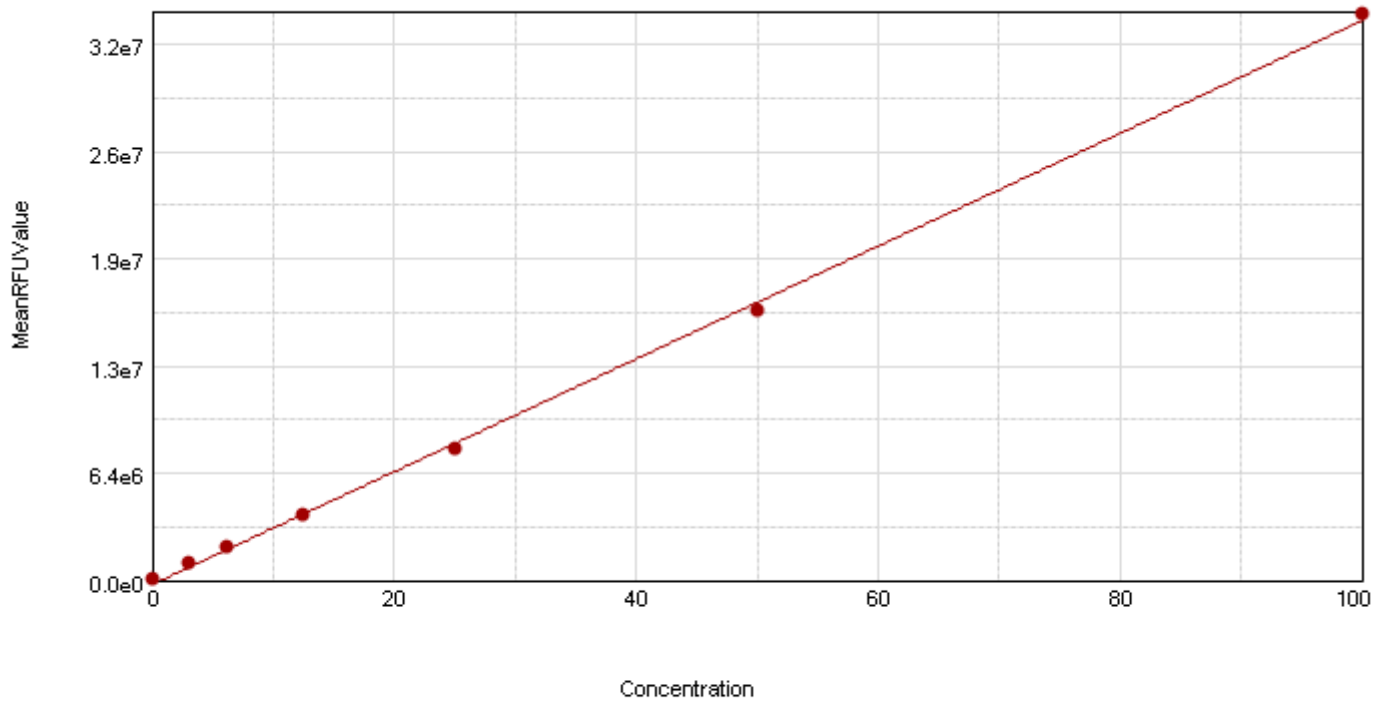
Unknowns_NoDiln

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
01	A1	2896412.000	9.301	9.301	0.000	0.0
02	B1	1285619.000	4.519	4.519	0.000	0.0
03	C1	5655719.000	17.494	17.494	0.000	0.0
04	D1	1282138.000	4.509	4.509	0.000	0.0
05	E1	1289536.000	4.531	4.531	0.000	0.0
06	F1	6481178.000	19.944	19.944	0.000	0.0
07	G1	7025570.000	21.561	21.561	0.000	0.0
08	H1	5293991.000	16.420	16.420	0.000	0.0
09	A2	5391326.000	16.709	16.709	0.000	0.0
10	B2	2495200.000	8.110	8.110	0.000	0.0
11	C2	8297129.000	25.336	25.336	0.000	0.0
12	D2	3861326.000	12.166	12.166	0.000	0.0
13	E2	1084530.000	3.922	3.922	0.000	0.0
14	F2	5900147.000	18.219	18.219	0.000	0.0
15	G2	1330998.000	4.654	4.654	0.000	0.0
16	H2	537667.000	2.298	2.298	0.000	0.0
17	A3	5622580.000	17.395	17.395	0.000	0.0
18	B3	3537858.000	11.206	11.206	0.000	0.0
19	C3	1817934.000	6.099	6.099	0.000	0.0
20	D3	2074148.000	6.860	6.860	0.000	0.0
21	E3	1849058.000	6.192	6.192	0.000	0.0
22	F3	2613597.000	8.462	8.462	0.000	0.0
23	G3	1481837.000	5.102	5.102	0.000	0.0
24	H3	6010019.000	18.545	18.545	0.000	0.0
25	A4	9910609.000	30.126	30.126	0.000	0.0
26	B4	3283875.000	10.452	10.452	0.000	0.0
27	C4	7152289.000	21.937	21.937	0.000	0.0
28	D4	1609601.000	5.481	5.481	0.000	0.0
29	E4	971516.000	3.587	3.587	0.000	0.0
30	F4	1452217.000	5.014	5.014	0.000	0.0
31	G4	6098630.000	18.809	18.809	0.000	0.0
32	H4	1207070.000	4.286	4.286	0.000	0.0
33	A5	2299342.000	7.529	7.529	0.000	0.0
34	B5	1884910.000	6.298	6.298	0.000	0.0
35	C5	72551.000	0.918	0.918	0.000	0.0
36	D5	667683.000	2.684	2.684	0.000	0.0
37	E5	1118488.000	4.023	4.023	0.000	0.0
38	F5	971342.000	3.586	3.586	0.000	0.0
39	G5	527178.000	2.267	2.267	0.000	0.0
40	H5	68509.000	0.906	0.906	0.000	0.0
41	A6	981522.000	3.616	3.616	0.000	0.0
42	B6	1423612.000	4.929	4.929	0.000	0.0
43	C6	1255752.000	4.430	4.430	0.000	0.0
44	D6	1169036.000	4.173	4.173	0.000	0.0
45	E6	1936301.000	6.451	6.451	0.000	0.0
46	F6	448394.000	2.033	2.033	0.000	0.0
47	G6	2187627.000	7.197	7.197	0.000	0.0
48	H6	958976.000	3.549	3.549	0.000	0.0
49	A7	2777664.000	8.949	8.949	0.000	0.0
50	B7	1203875.000	4.276	4.276	0.000	0.0
51	C7	1587043.000	5.414	5.414	0.000	0.0
52	D7	69113.000	0.907	0.907	0.000	0.0
53	E7	1428334.000	4.943	4.943	0.000	0.0
54	F7	369177.000	1.798	1.798	0.000	0.0
55	G7	1233074.000	4.363	4.363	0.000	0.0
56	H7	3526190.000	11.171	11.171	0.000	0.0
57	A8	3076171.000	9.835	9.835	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
58	B8	1445000.000	4.992	4.992	0.000	0.0
59	C8	1730987.000	5.841	5.841	0.000	0.0
60	D8	722443.000	2.847	2.847	0.000	0.0
61	E8	3624954.000	11.464	11.464	0.000	0.0
62	F8	465699.000	2.085	2.085	0.000	0.0
63	G8	739218.000	2.897	2.897	0.000	0.0
64	H8	397561.000	1.883	1.883	0.000	0.0
65	A9	896363.000	3.363	3.363	0.000	0.0
66	B9	2339389.000	7.648	7.648	0.000	0.0
67	C9	459039.000	2.065	2.065	0.000	0.0
68	D9	3287285.000	10.462	10.462	0.000	0.0
69	E9	3461119.000	10.978	10.978	0.000	0.0
70	F9	3642298.000	11.516	11.516	0.000	0.0
71	G9	290443.000	1.564	1.564	0.000	0.0
72	H9	1474703.000	5.080	5.080	0.000	0.0
73	A10	1400420.000	4.860	4.860	0.000	0.0
74	B10	3547931.000	11.236	11.236	0.000	0.0
75	C10	1617878.000	5.506	5.506	0.000	0.0
76	D10	1866611.000	6.244	6.244	0.000	0.0
77	E10	1002148.000	3.677	3.677	0.000	0.0
78	F10	1673746.000	5.671	5.671	0.000	0.0
79	G10	1604054.000	5.464	5.464	0.000	0.0
80	H10	507251.000	2.208	2.208	0.000	0.0
81	A11	571359.000	2.398	2.398	0.000	0.0
82	B11	1170655.000	4.178	4.178	0.000	0.0
83	C11	1640658.000	5.573	5.573	0.000	0.0
84	D11	810689.000	3.109	3.109	0.000	0.0
85	E11	4907389.000	15.272	15.272	0.000	0.0
86	F11	1207599.000	4.287	4.287	0.000	0.0
87	G11	3225021.000	10.277	10.277	0.000	0.0
88	H11	997392.000	3.663	3.663	0.000	0.0

Standard Curve



● STD#1 (Standards: MeanRF... vs Concentr...)

Curve Fit Results ▼

Intro

Quantitation of double-stranded DNA using Quant-iT PicoGreen Reagent

Invitrogen (Molecular Probes)

MATERIALS

- o Quant-iT PicoGreen dsDNA Assay Kit, including lambda DNA standard (Invitrogen cat. #P7589 or P11496)
- o Black 96-well plate (Greiner Bio-One, cat. # 655096)
- o Brown or amber (light-blocking) microcentrifuge tubes

METHODS*Set up the protocol:*

- o Select Wells to Read and Assay Plate Type by clicking on "Settings" and locating the options on the left side of the screen.
- o Click the Template button to open a window where you can assign wells of the microplate to pre-set template groups using the drop-down menu to select the appropriate template group. There are preconfigured template groups in the PicoGreen Fluorescence protocol including Standards, Unknowns, and Unknowns_NoDiln (for undiluted samples). Assigning wells to pre-set template groups populates group tables in the protocol with the corresponding data acquired when the microplate is read.

Prepare the assay

The method for this assay follows the instructions in the product information sheet for Quant-iT PicoGreen dsDNA Reagent and Kits from Molecular Probes, except that the assay volume is proportionately reduced from 2.0 mL to 200 μ L to fit a 96-well microplate format.

- o Prepare 1X TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) by diluting the concentrated buffer from the kit 20-fold with distilled DNase-free water, as required by Molecular Probes.
- o Prepare an aqueous working solution of Quant-iT PicoGreen reagent by making a 200-fold dilution of the concentrated DMSO solution in TE buffer (prepared above). Preparation of the solution in a plastic container, rather than glass, is recommended, as the reagent may adsorb to glass surfaces. Protect the solution from light by using amber or brown tubes, or by covering with foil. This solution should be used within a few hours of its preparation.
- o DNA standard curve: Prepare a 2 μ g/mL stock solution of dsDNA in TE. The lambda DNA standard provided with the kit can be diluted 50-fold in TE to make the 2 μ g/mL solution. Note: in some cases it may be preferable to make the standard curve using DNA similar to the type being assayed.
- o A high-range standard curve may be prepared from 1 ng/mL to 1 μ g/mL, or a low-range standard curve may be prepared from 25 μ g/mL to 25 ng/mL. For the high-range curve, follow the dilution scheme shown in the PicoGreen product insert; for the low-range curve, dilute the 2 μ g/mL solution 40-fold to yield a 50 ng/mL solution, and refer to the alternative dilution scheme in the product insert.
- o Pipet standards into a solid black 96-well microplate at 100 μ L per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).
- o Add 100 μ L of the aqueous working solution of Quant-iT PicoGreen reagent to each well. Mix well by trituration or plate shaker and incubate for 2 to 5 minutes at room temperature, protected from light.

Read the microplate

- " Make sure the purple plate adapter is in the microplate reader drawer. Place the microplate in the drawer.
- " Click the Read button in the SoftMax Pro software. The instrument will read the plate and the relative fluorescence units will be displayed in the Plate section of the protocol.

Analyze the data

- o After the microplate has been read, the relative fluorescence units (RFUs) will be displayed in the Plate section. The data will be analyzed in the Group Tables that were created when the template was set up.
- o Standards assigned in the Template (and thus displayed in the Standards group table) will be automatically plotted in the Standard Curve section of the protocol. A linear curve fit is applied by default, but a log-log fit may be used when plotting a standard curve over a wide dynamic range. Curve fits are chosen from the drop-down Curve Fit menu in the graph section's tool bar.

READER SUITABILITY:

All SpectraMax readers with fluorescence capability.

PROTOCOL REVISION HISTORY:

- v 1.1: Imported from SMP 5.4.2 April 2011 (CLO & ELM)
- v 1.2: Emission wavelength changed from 540 nm to 525 nm. (CLO)

Plate02

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.0e6	5.8e6	6.1e6	1.0e7	2.3e6	1.0e6	2.9e6	3.2e6	9.3e5	1.4e6	6.1e5	3.3e7
B	1.4e6	2.6e6	3.8e6	3.4e6	2.0e6	1.5e6	1.2e6	1.5e6	2.4e6	3.8e6	1.2e6	1.6e7
C	6.0e6	8.9e6	1.9e6	8.0e6	7.4e4	1.3e6	1.6e6	1.8e6	4.7e5	1.6e6	1.6e6	8.6e6
D	1.4e6	4.0e6	2.2e6	1.7e6	7.1e5	1.2e6	1.6e5	7.5e5	3.3e6	1.9e6	5.2e5	3.9e6
E	1.4e6	1.1e6	2.0e6	1.0e6	1.1e6	1.9e6	1.4e6	3.8e6	3.6e6	9.9e5	5.4e6	2.0e6
F	6.5e6	5.8e6	2.8e6	1.4e6	9.9e5	4.5e5	3.8e5	4.6e5	3.8e6	1.7e6	1.2e6	1.1e6
G	7.4e6	1.3e6	1.5e6	6.3e6	5.4e5	2.2e6	1.3e6	7.4e5	3.2e5	1.7e6	3.5e6	6.7e4
H	5.5e6	5.3e5	6.0e6	1.2e6	7.0e4	9.9e5	3.5e6	3.5e5	1.5e6	5.1e5	9.8e5	6.6e4

Settings Information

Endpoint
 Fluorescence
 Lm1 485, 535
 Slide(s) Ex1, Em1
 More Settings
 Shake Off
 ReadOrder Row
 Show Optimizer On
 PMT and Optics
 Integration Time 400 ms
 Read from Top
 Read Height 1.00 mm

Read Information

FilterMax F5
 ROM vV1.1 b32 10.12.2010
 Start Read : 3:39 PM
 3/17/2014

Mean Temperature : 29.5 °C

Reduction Settings

Wavelength Combination : !Lm1

Standards

Sample	Concentration	BackCalcConc	Wells	RFU_Values	MeanRFUValue	SD	CV
1	100.000	100.554	A12	32889774.0...	32889774.000	0....	0.0
2	50.000	48.342	B12	15807006.0...	15807006.000	0....	0.0
3	25.000	26.387	C12	8623534.000	8623534.000	0....	0.0
4	12.500	11.978	D12	3909196.000	3909196.000	0....	0.0
5	6.250	6.049	E12	1969376.000	1969376.000	0....	0.0
6	3.125	3.332	F12	1080313.000	1080313.000	0....	0.0
7	0.000	0.234	G12	66837.000	66457.500	53...	0.8
		0.232	H12	66078.000			

Unknowns

Sample	Wells	RFU_Values	Concentration	Mean_Conc	SD	CV	Dilution	AdjConc
--------	-------	------------	---------------	-----------	----	----	----------	---------

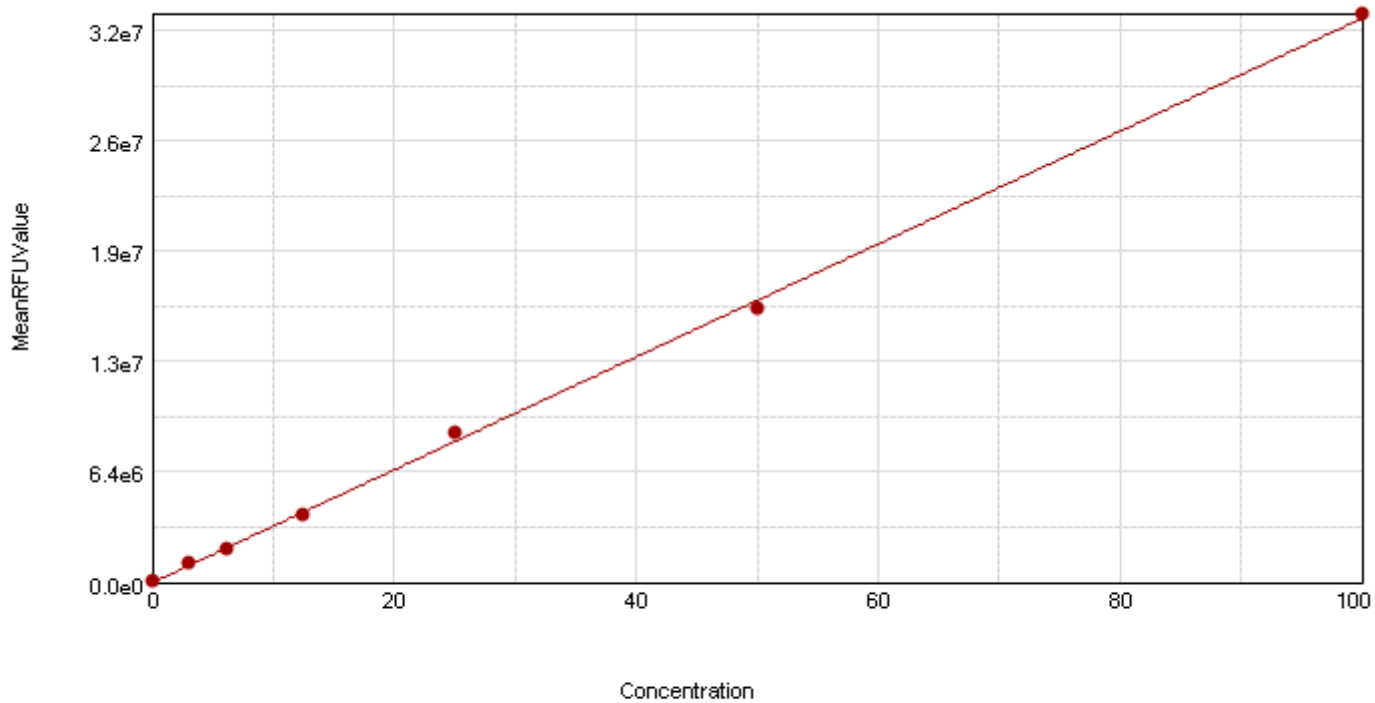
Unknowns_NoDiln

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
01	A1	3047981.000	9.346	9.346	0.000	0.0
02	B1	1368708.000	4.213	4.213	0.000	0.0
03	C1	6032821.000	18.469	18.469	0.000	0.0
04	D1	1351071.000	4.159	4.159	0.000	0.0
05	E1	1372666.000	4.225	4.225	0.000	0.0
06	F1	6475320.000	19.821	19.821	0.000	0.0
07	G1	7366412.000	22.545	22.545	0.000	0.0
08	H1	5492624.000	16.818	16.818	0.000	0.0
09	A2	5817680.000	17.811	17.811	0.000	0.0
10	B2	2589877.000	7.946	7.946	0.000	0.0
11	C2	8879726.000	27.170	27.170	0.000	0.0
12	D2	3978049.000	12.188	12.188	0.000	0.0
13	E2	1140609.000	3.516	3.516	0.000	0.0
14	F2	5849614.000	17.909	17.909	0.000	0.0
15	G2	1339965.000	4.126	4.126	0.000	0.0
16	H2	534635.000	1.664	1.664	0.000	0.0
17	A3	6091096.000	18.647	18.647	0.000	0.0
18	B3	3846218.000	11.786	11.786	0.000	0.0
19	C3	1920843.000	5.901	5.901	0.000	0.0
20	D3	2197037.000	6.745	6.745	0.000	0.0
21	E3	1955896.000	6.008	6.008	0.000	0.0
22	F3	2751151.000	8.439	8.439	0.000	0.0
23	G3	1542465.000	4.744	4.744	0.000	0.0
24	H3	6037978.000	18.484	18.484	0.000	0.0
25	A4	10388590.000	31.782	31.782	0.000	0.0
26	B4	3430626.000	10.515	10.515	0.000	0.0
27	C4	7976434.000	24.409	24.409	0.000	0.0
28	D4	1684456.000	5.178	5.178	0.000	0.0
29	E4	1029815.000	3.178	3.178	0.000	0.0
30	F4	1441495.000	4.436	4.436	0.000	0.0
31	G4	6274378.000	19.207	19.207	0.000	0.0
32	H4	1224135.000	3.772	3.772	0.000	0.0
33	A5	2349703.000	7.212	7.212	0.000	0.0
34	B5	1956793.000	6.011	6.011	0.000	0.0
35	C5	73769.000	0.256	0.256	0.000	0.0
36	D5	708867.000	2.197	2.197	0.000	0.0
37	E5	1127721.000	3.477	3.477	0.000	0.0
38	F5	990785.000	3.058	3.058	0.000	0.0
39	G5	542721.000	1.689	1.689	0.000	0.0
40	H5	70317.000	0.245	0.245	0.000	0.0
41	A6	1026447.000	3.167	3.167	0.000	0.0
42	B6	1450935.000	4.465	4.465	0.000	0.0
43	C6	1317557.000	4.057	4.057	0.000	0.0
44	D6	1184925.000	3.652	3.652	0.000	0.0
45	E6	1936388.000	5.948	5.948	0.000	0.0
46	F6	448681.000	1.401	1.401	0.000	0.0
47	G6	2209371.000	6.783	6.783	0.000	0.0
48	H6	993289.000	3.066	3.066	0.000	0.0
49	A7	2854850.000	8.756	8.756	0.000	0.0
50	B7	1221383.000	3.763	3.763	0.000	0.0
51	C7	1619495.000	4.980	4.980	0.000	0.0
52	D7	162079.000	0.525	0.525	0.000	0.0
53	E7	1390716.000	4.281	4.281	0.000	0.0
54	F7	375710.000	1.178	1.178	0.000	0.0
55	G7	1280047.000	3.942	3.942	0.000	0.0
56	H7	3473398.000	10.646	10.646	0.000	0.0
57	A8	3244223.000	9.946	9.946	0.000	0.0

Unknowns_NoDiln (Contd)

Sample	Wells	RFU_Values	Concentration	MeanConc	SD	CV
58	B8	1499048.000	4.612	4.612	0.000	0.0
59	C8	1781148.000	5.474	5.474	0.000	0.0
60	D8	750900.000	2.325	2.325	0.000	0.0
61	E8	3786311.000	11.602	11.602	0.000	0.0
62	F8	464619.000	1.450	1.450	0.000	0.0
63	G8	735146.000	2.277	2.277	0.000	0.0
64	H8	350483.000	1.101	1.101	0.000	0.0
65	A9	932108.000	2.879	2.879	0.000	0.0
66	B9	2366345.000	7.263	7.263	0.000	0.0
67	C9	473034.000	1.476	1.476	0.000	0.0
68	D9	3305616.000	10.133	10.133	0.000	0.0
69	E9	3561907.000	10.917	10.917	0.000	0.0
70	F9	3823855.000	11.717	11.717	0.000	0.0
71	G9	321119.000	1.012	1.012	0.000	0.0
72	H9	1468622.000	4.519	4.519	0.000	0.0
73	A10	1442282.000	4.438	4.438	0.000	0.0
74	B10	3833652.000	11.747	11.747	0.000	0.0
75	C10	1597519.000	4.913	4.913	0.000	0.0
76	D10	1895845.000	5.825	5.825	0.000	0.0
77	E10	990576.000	3.058	3.058	0.000	0.0
78	F10	1692356.000	5.203	5.203	0.000	0.0
79	G10	1695695.000	5.213	5.213	0.000	0.0
80	H10	509825.000	1.588	1.588	0.000	0.0
81	A11	608148.000	1.889	1.889	0.000	0.0
82	B11	1226696.000	3.779	3.779	0.000	0.0
83	C11	1643193.000	5.052	5.052	0.000	0.0
84	D11	518411.000	1.615	1.615	0.000	0.0
85	E11	5385821.000	16.491	16.491	0.000	0.0
86	F11	1201116.000	3.701	3.701	0.000	0.0
87	G11	3509041.000	10.755	10.755	0.000	0.0
88	H11	976748.000	3.015	3.015	0.000	0.0

Standard Curve



● STD#1 (Standards: MeanRF... vs Concentr...)

Curve Fit Results ▼