﻿##BLOCKS= 4

Note:

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 uL 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

o 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 µg/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 µg/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 µg/mL, or a low-range standard curve may be prepared from 25 pg/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 µg/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 µL per well, preferably in triplicate. Be sure to include a set of buffer blank wells containing TE only (no DNA).

o Add 100 µL 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.

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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)

~End

Plate: Plate01 1.3 TimeFormat Endpoint Fluorescence FALSE Raw FALSE 1 1 525 1 12 96 490 Manual 515 6 1 8

Temperature(¡C) A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12 F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12 H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12

18.8 4179.76 761.665 2034.78 1058.21 1002.245 322.552 266.474 718.68 1690.287 2000.466 7.992 9.176 415.366 271.336 803.755 1555.552 1061.94 1135.523 643.624 718.138 2002.82 25.028 32.6 24.99 47.866 1815.806 1103.625 2603.331 244.664 2561.99 841.666 946.095 2180.295 12.215 16.756 5.225 5.92 626.464 592.596 2060.313 1323.118 2851.966 1759.28 769.24 2642.333 16.97 10.747 0.65 4336.852 573.364 607.318 1037.504 175.979 2907.847 985.989 1082.764 2145.295 11.244 27.576 0.562 405.821 515.983 812.923 1376.317 376.118 2286.356 1148.637 902.769 2735.687 16.685 25.604 0.607 44.46 1116.393 303.709 3117.646 652.143 1167.677 950.113 470.181 1205.073 13.244 9.67 0.679 3.478 664.54 2500.108 1335.558 1193.831 556.125 1864.365 1861.211 2425.729 8.441 18.275 0.699

A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 B1 B2 B3 B4 B5 B6 B7 B8 B9 B10 B11 B12 C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 E1 E2 E3 E4 E5 E6 E7 E8 E9 E10 E11 E12 F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11 G12 H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12

4179.76 761.665 2034.78 1058.21 1002.245 322.552 266.474 718.68 1690.287 2000.466 7.992 9.176 415.366 271.336 803.755 1555.552 1061.94 1135.523 643.624 718.138 2002.82 25.028 32.6 24.99 47.866 1815.806 1103.625 2603.331 244.664 2561.99 841.666 946.095 2180.295 12.215 16.756 5.225 5.92 626.464 592.596 2060.313 1323.118 2851.966 1759.28 769.24 2642.333 16.97 10.747 0.65 4336.852 573.364 607.318 1037.504 175.979 2907.847 985.989 1082.764 2145.295 11.244 27.576 0.562 405.821 515.983 812.923 1376.317 376.118 2286.356 1148.637 902.769 2735.687 16.685 25.604 0.607 44.46 1116.393 303.709 3117.646 652.143 1167.677 950.113 470.181 1205.073 13.244 9.67 0.679 3.478 664.54 2500.108 1335.558 1193.831 556.125 1864.365 1861.211 2425.729 8.441 18.275 0.699

~End

Group: Standards

Sample Concentration BackCalcConc Wells RFU\_Values MeanRFUValue SD CV

01 1.000 0.982 A1 4179.760 4258.306 111.081 2.6

1.019 E1 4336.852

02 0.100 0.098 B1 415.366 410.594 6.749 1.6

0.096 F1 405.821

03 0.010 0.012 C1 47.866 46.163 2.408 5.2

0.011 G1 44.460

04 0.000 0.002 D1 5.920 4.699 1.727 36.7

0.001 H1 3.478

Group Summaries

~End

Group: Unknowns

Sample Wells RFU\_Values Concentration Mean\_Conc SD CV Dilution AdjConc

01 A2 761.665 0.179 0.179 0.000 0.0 200.0 35.858

02 B2 271.336 0.064 0.064 0.000 0.0 200.0 12.832

03 C2 1815.806 0.427 0.427 0.000 0.0 200.0 85.360

04 D2 626.464 0.148 0.148 0.000 0.0 200.0 29.509

05 E2 573.364 0.135 0.135 0.000 0.0 200.0 27.015

06 F2 515.983 0.122 0.122 0.000 0.0 200.0 24.321

07 G2 1116.393 0.263 0.263 0.000 0.0 200.0 52.516

08 H2 664.540 0.156 0.156 0.000 0.0 200.0 31.297

09 A3 2034.780 0.478 0.478 0.000 0.0 200.0 95.643

10 B3 803.755 0.189 0.189 0.000 0.0 200.0 37.834

11 C3 1103.625 0.260 0.260 0.000 0.0 200.0 51.916

12 D3 592.596 0.140 0.140 0.000 0.0 200.0 27.918

13 E3 607.318 0.143 0.143 0.000 0.0 200.0 28.610

14 F3 812.923 0.191 0.191 0.000 0.0 200.0 38.265

15 G3 303.709 0.072 0.072 0.000 0.0 200.0 14.352

16 H3 2500.108 0.587 0.587 0.000 0.0 200.0 117.495

17 A4 1058.210 0.249 0.249 0.000 0.0 200.0 49.784

18 B4 1555.552 0.366 0.366 0.000 0.0 200.0 73.139

19 C4 2603.331 0.612 0.612 0.000 0.0 200.0 122.343

20 D4 2060.313 0.484 0.484 0.000 0.0 200.0 96.842

21 E4 1037.504 0.244 0.244 0.000 0.0 200.0 48.811

22 F4 1376.317 0.324 0.324 0.000 0.0 200.0 64.722

23 G4 3117.646 0.732 0.732 0.000 0.0 200.0 146.495

24 H4 1335.558 0.314 0.314 0.000 0.0 200.0 62.808

25 A5 1002.245 0.236 0.236 0.000 0.0 200.0 47.155

26 B5 1061.940 0.250 0.250 0.000 0.0 200.0 49.959

27 C5 244.664 0.058 0.058 0.000 0.0 200.0 11.579

28 D5 1323.118 0.311 0.311 0.000 0.0 200.0 62.224

29 E5 175.979 0.042 0.042 0.000 0.0 200.0 8.354

30 F5 376.118 0.089 0.089 0.000 0.0 200.0 17.752

31 G5 652.143 0.154 0.154 0.000 0.0 200.0 30.715

32 H5 1193.831 0.281 0.281 0.000 0.0 200.0 56.152

33 A6 322.552 0.076 0.076 0.000 0.0 200.0 15.237

34 B6 1135.523 0.267 0.267 0.000 0.0 200.0 53.414

35 C6 2561.990 0.602 0.602 0.000 0.0 200.0 120.401

36 D6 2851.966 0.670 0.670 0.000 0.0 200.0 134.018

37 E6 2907.847 0.683 0.683 0.000 0.0 200.0 136.643

38 F6 2286.356 0.537 0.537 0.000 0.0 200.0 107.457

39 G6 1167.677 0.275 0.275 0.000 0.0 200.0 54.924

40 H6 556.125 0.131 0.131 0.000 0.0 200.0 26.206

41 A7 266.474 0.063 0.063 0.000 0.0 200.0 12.604

42 B7 643.624 0.152 0.152 0.000 0.0 200.0 30.315

43 C7 841.666 0.198 0.198 0.000 0.0 200.0 39.615

44 D7 1759.280 0.414 0.414 0.000 0.0 200.0 82.706

45 E7 985.989 0.232 0.232 0.000 0.0 200.0 46.392

46 F7 1148.637 0.270 0.270 0.000 0.0 200.0 54.030

47 G7 950.113 0.224 0.224 0.000 0.0 200.0 44.707

48 H7 1864.365 0.438 0.438 0.000 0.0 200.0 87.641

49 A8 718.680 0.169 0.169 0.000 0.0 200.0 33.839

50 B8 718.138 0.169 0.169 0.000 0.0 200.0 33.814

51 C8 946.095 0.223 0.223 0.000 0.0 200.0 44.519

52 D8 769.240 0.181 0.181 0.000 0.0 200.0 36.213

53 E8 1082.764 0.255 0.255 0.000 0.0 200.0 50.937

54 F8 902.769 0.212 0.212 0.000 0.0 200.0 42.484

55 G8 470.181 0.111 0.111 0.000 0.0 200.0 22.170

56 H8 1861.211 0.437 0.437 0.000 0.0 200.0 87.493

57 A9 1690.287 0.397 0.397 0.000 0.0 200.0 79.466

58 B9 2002.820 0.471 0.471 0.000 0.0 200.0 94.142

59 C9 2180.295 0.512 0.512 0.000 0.0 200.0 102.477

60 D9 2642.333 0.621 0.621 0.000 0.0 200.0 124.174

61 E9 2145.295 0.504 0.504 0.000 0.0 200.0 100.833

62 F9 2735.687 0.643 0.643 0.000 0.0 200.0 128.558

63 G9 1205.073 0.283 0.283 0.000 0.0 200.0 56.680

64 H9 2425.729 0.570 0.570 0.000 0.0 200.0 114.002

65 A10 2000.466 0.470 0.470 0.000 0.0 200.0 94.032

66 B10 25.028 0.006 0.006 0.000 0.0 200.0 1.265

67 C10 12.215 0.003 0.003 0.000 0.0 200.0 0.664

68 D10 16.970 0.004 0.004 0.000 0.0 200.0 0.887

69 E10 11.244 0.003 0.003 0.000 0.0 200.0 0.618

70 F10 16.685 0.004 0.004 0.000 0.0 200.0 0.873

71 G10 13.244 0.004 0.004 0.000 0.0 200.0 0.712

72 H10 8.441 0.002 0.002 0.000 0.0 200.0 0.486

73 A11 7.992 0.002 0.002 0.000 0.0 200.0 0.465

74 B11 32.600 0.008 0.008 0.000 0.0 200.0 1.621

75 C11 16.756 0.004 0.004 0.000 0.0 200.0 0.877

76 D11 10.747 0.003 0.003 0.000 0.0 200.0 0.595

77 E11 27.576 0.007 0.007 0.000 0.0 200.0 1.385

78 F11 25.604 0.006 0.006 0.000 0.0 200.0 1.292

79 G11 9.670 0.003 0.003 0.000 0.0 200.0 0.544

80 H11 18.275 0.005 0.005 0.000 0.0 200.0 0.948

81 A12 9.176 0.003 0.003 0.000 0.0 200.0 0.521

82 B12 24.990 0.006 0.006 0.000 0.0 200.0 1.263

83 C12 5.225 0.002 0.002 0.000 0.0 200.0 0.335

84 D12 0.650 0.001 0.001 0.000 0.0 200.0 0.120

85 E12 0.562 0.001 0.001 0.000 0.0 200.0 0.116

86 F12 0.607 0.001 0.001 0.000 0.0 200.0 0.118

87 G12 0.679 0.001 0.001 0.000 0.0 200.0 0.122

88 H12 0.699 0.001 0.001 0.000 0.0 200.0 0.123

Group Summaries

~End

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