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Tech Note

Qubit Assay using Synergy™ Neo2

Introduction

The Qubit dsDNA BR Assay is currently used in a large number of laboratories for DNA quantification; however the kit is designed for a low-throughput, microcentrifuge tube format. Therefore, laboratories looking for increased throughput are adapting the reagent for use in a 96-well microplate assay format. This Tech Note describes the materials, methods and Gen5™ parameters required to establish a standard curve and perform a DNA quantification with the Qubit dsDNA BR assay reagent in a 96-well plate format using a Synergy Neo2 Multi-Mode Reader. The minimal number of standards required to accurately model the range for the Qubit reagent (0.1 – 1000 ng/µL) is determined to be four: 0.1, 1, 10 and 1,000 ng/µL.

Materials Used:

- Qubit® dsDNA BR Assay Kit (Molecular Probes Catalog # Q32850)
- Purified Herring Sperm DNA (Sigma-Aldrich, Catalog # D6898) in TE Buffer
- TE Buffer (10 mM Tris, 1 mM EDTA, pH 8.0).
- Solid black, 96-well microplates (Corning, Catalog # 3915)
- Single- and 8-channel pipettor with 10-200 µL range

Assay Setup:

- The Qubit working solution was prepared by diluting the Qubit BR Reagent (200X) 1:200 in Qubit dsDNA BR Buffer.
- A 4-, 5-, 6- and 8-point serial log dilution series of standards ranging between 1000 ng/µL and 0.1 ng/µL was created from a 2.2 mg/mL stock solution of Herring Sperm DNA in TE buffer. This is the stated validated range for the Qubit dsDNA Assay Kit.
- The actual concentration of each standard was verified in a Synergy Neo2 via A_{260} read in a 96-well plate with path length correction engaged.
- A multi-channel pipettor was used to add 10 µL of DNA standard to the 96-well plate, followed by 190 µL of Qubit working solution (for a final volume of 200 µL) and incubated for 5 min at RT, then read on a Synergy Neo2.

Synergy Neo2 Setup:

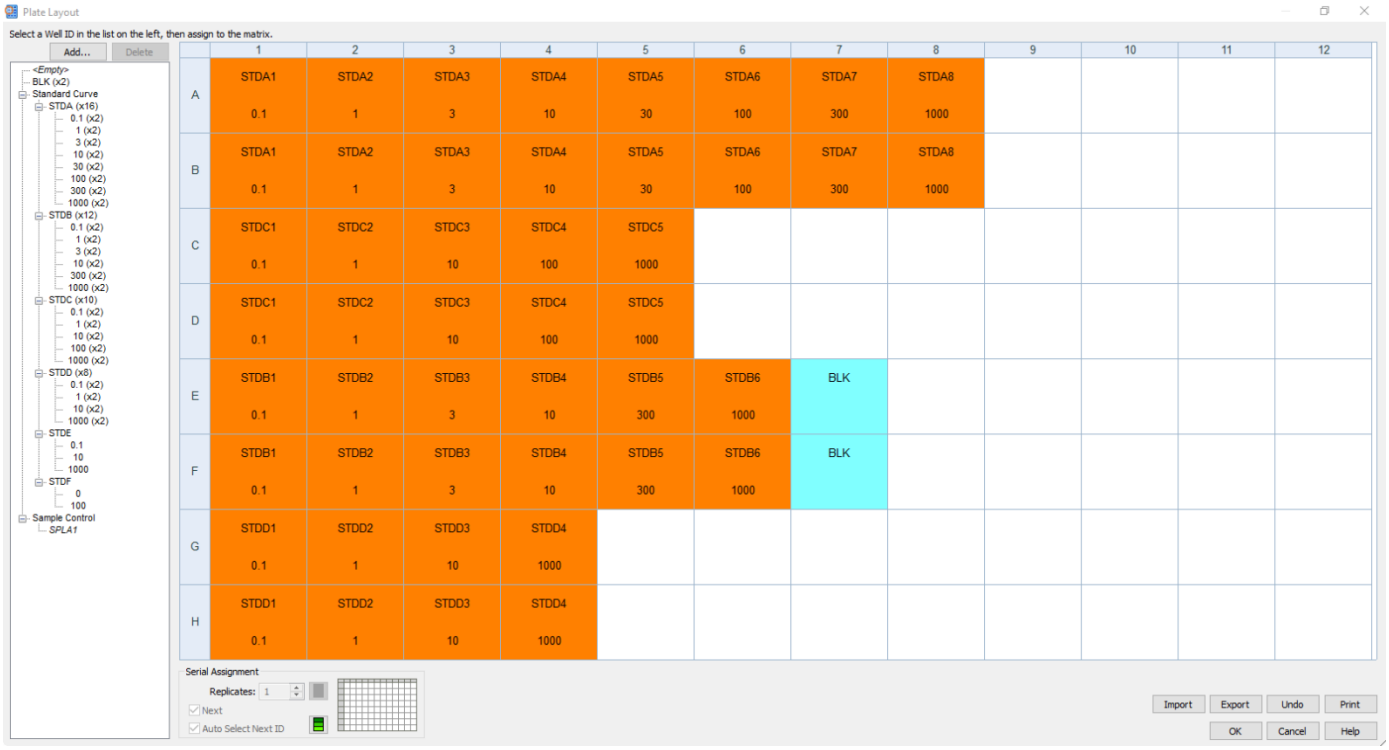
- A Corning #3915 black bottom, 96-well microplate is used for this assay, however the default “96 WELL PLATE” can be selected as the Plate Type.
- Fluorescence Intensity is measured using a single top PMT with GFP filter cube (Part # 1035108; EX 485/20 nm EM 530/25, DM 510).

Gen5 Recommended Protocol Setup:

Procedure

1. Select the 96-well plate from the Plate Type dropdown menu
2. Read Method:
    - Detection Method: Fluorescence Intensity
    - Read Type: Endpoint/Kinetic
    - Optics Type: Filters
3. Read Step: Fluorescence Intensity
    - Step Label: dsDNA Std Crv
    - PMT: Single
    - Filter Set 1: 485/528
    - Optics Position: Top
    - Gain: 50

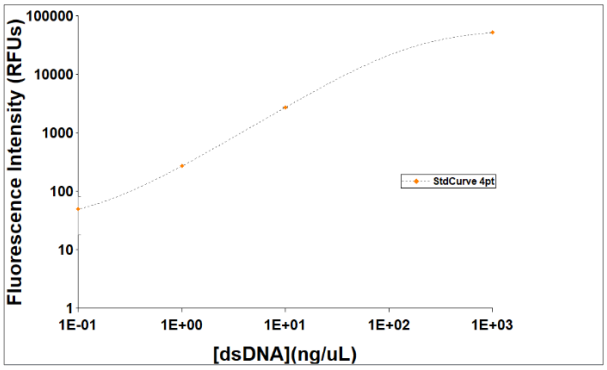
Plate Layout

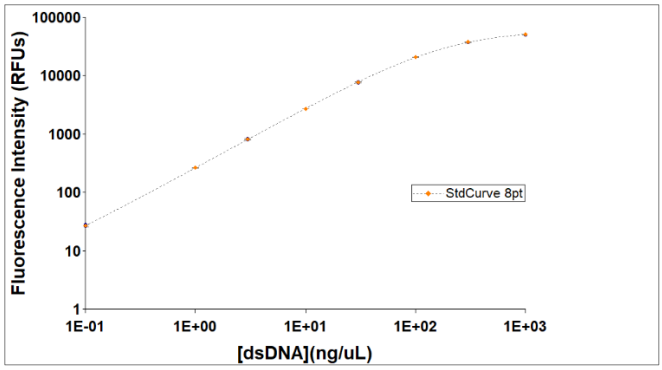


Data Reduction

Transformation	
Type	Blank
Data in	dsDNA Std Crv: 485/20, 528/20
Blank Wells	BLK
New Dataset Name	Blanked dsDNA Std Crv: 485/20, 528/20
Formula	X-BLK
Curve Analysis	
Type	Standard Curve
Data In:	
Well ID	STD
X Axis Data	<Concentrations/Dilutions>
Y Axis Data	Blank dsDNA Std Crv: 485/20, 528/20
Downsize Image	50%
Curve Fit:	
Method	4-parameter Nonlinear Regression
Axis Presentation	Log (both X and Y)
Y Axis Data	Use average of replicates
	Exploration factor: 1.1
Parameter Constraints	None
Formula	Y = (A-D)/(1+(X/C)^B) + D
Data Output Name	[DNA]

Results





The composite data was plotted in Gen5™ for standard curves with varying number of standards, 4 and 8 standards. The plot shows excellent correlation between standard curves fit using a 4-parameter nonlinear regression analysis generated with as little as four standard concentrations; 0.1, 1.0, 10 and 1,000 ng/µL dsDNA.

Conclusion

The use of fluorescent reagents can increase the specificity and sensitivity of nucleic acid quantification. The use of Qubit dsDNA BR reagent in conjunction with a Synergy™ Neo2 Multi-Mode Reader allows dsDNA quantitation with as few as four (4) DNA standards in a 96-well microplate format. The curve fit produced with 4 standards is comparable to that of standard curves produced with as many as eight (8) standards allowing significant reagent savings.