# Sybr® Green I dsDNA Assay

## Introduction

The Sybr® Green I dye is a fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA). Used in conjunction with the micro-volume capability of the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer, the Sybr® Green I assay provides a highly sensitive means of dsDNA quantitation with minimal consumption of sample. The main disadvantage of general UV spectroscopy for dsDNA quantitation is the contribution of signal from single-stranded DNA (ssDNA) and other contaminants, such as protein and extraction buffers. Sybr® Green I reagent circumvents such contributions from interfering substances by exhibiting an emission maximum at 527nm when bound to dsDNA (unbound Sybr® Green I reagent exhibits minimal fluorescence in solution). The ability of the NanoDrop 3300 to measure as little as 1 ul of sample, allows significantly scaled-down reaction volumes, thereby using only a fraction of sample commonly needed for conventional cuvette-based fluorometers. The NanoDrop 3300 has demonstrated a detection range for dsDNA bound with Sybr® Green I reagent of 1 ng/ml– 1000 ng/ml and has proven to be 75 times more sensitive than using the Hoechst 33258 dye with this system. Readings taken at the lowest detection limit consume only 2 picograms of dsDNA per measurement.

## Assay Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 2uL pipettor
- Low retention nuclease free pipette tips

Materials:

- Low lint laboratory wipes
- Nuclease free sterile amber or foil covered 1.5 mL polypropylene tubes
- Nuclease free sterile clear 1.5 mL polypropylene tubes
- 10 uL sterile nuclease free low retention tips

Reagents:

- SYBR® Green I nucleic acid gel stain (Invitrogen Catalog Number - S-7567)
- Sterile Nuclease free 1X TE buffer pH 8.0 (10mM Tris, 1mM EDTA
- Control dsDNA

## Sybr® Green I Assay Protocol

This protocol is configured to provide enough volume for a maximum of five measurement replicates. This protocol has been observed to be effective for a final dye dilution in a range from 1:8000 to 1:10,000. As the dye intensity may be lot specific the investigator may change the dye dilution as necessary. The total reaction volume can also be scaled at the discretion of the investigator.

1. Dilute the Sybr® Green I reagent 1:100 in 1X TE pH 8.0. (This 1:100 dilution has shown to be stable for up to 6 hours at room temperature when protected from light.)

   Example: For a 1:100 dye dilution transfer 10 ul of 10,000 X concentrated Sybr® Green I dye and 990 ul of 1X TE buffer into a clean amber or foiled1.5ml microfuge tube and mix well with 1ml pipette.

2. Prepare a series of 2X serial dilutions of control dsDNA ranging from 2 ng/ml to 2,000 ng/ml in nuclease free tubes.

Example dilution series

| dsDNA [ng/ml] | dsDNA uL | 1X TE uL | Total Volume uL | *In assay dsDNA [ng/ml] |
|---|---|---|---|---|
| 2000.0 | | | 178.3 | 1000.0 |
| 1000.0 | 78.3 | 78.3 | 156.6 | 500.0 |
| 500.0 | 56.6 | 56.6 | 113.2 | 250.0 |
| 50.0 | 13.2 | 118.8 | 132.0 | 25.0 |
| 10.0 | 32.0 | 128.0 | 160.0 | 5.0 |
| 4.0 | 60.0 | 90.0 | 150.0 | 2.0 |
| 2.0 | 50.0 | 50.0 | 100 | 1.0 |

*In assay describes the final concentration of dsDNA that present in the reaction.

3. Prepare a working Sybr® Green I dilution ranging from 1:4,000 to 1:5,000 in a 1.5 ml amber or foiled nuclease free tube (specific dilution of the Sybr® Green I working solution may be lot specific).

**Example: For a 1:4,500 working solution dilute the 1:100 Sybr® Green I reagent by transferring 22.2 ul of 1:100 Sybr® Green I reagent and 977.7 ul of 1X TE pH 8.0 to an amber or foiled tube and mix well.

4. Record the dsDNA concentrations on each tube and add 5 ul of each standard dsDNA dilution to the respective amber tube. Centrifuge the samples briefly and transfer 5 ul of the Sybr® Green I working solution to the bottom of the tube and centrifuge briefly once again.

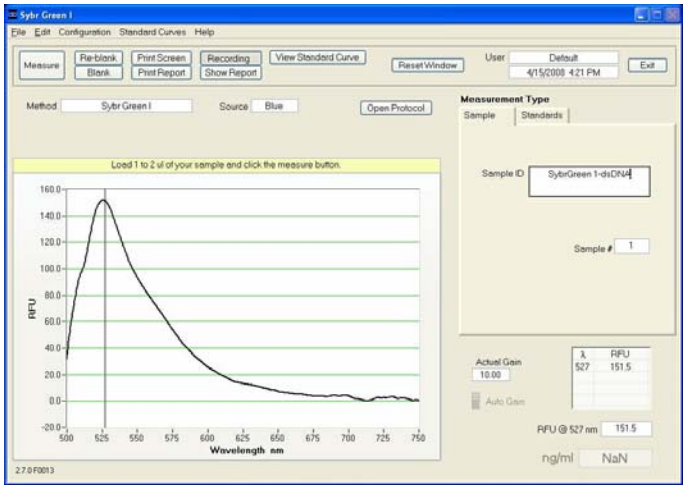
5. Add 5 ul of each unknown dsDNA sample into a labeled nuclease free amber tube, centrifuge the unknowns briefly, and add 5 ul of the diluted Sybr® Green I reagent to the bottom of the tube and centrifuge briefly once again.

6. Prepare a reference standard by adding 5 ul of 1X TE into a labeled nuclease free amber tube, centrifuge briefly, and add 5 ul of the diluted Sybr® Green I reagent to the bottom of the tube and centrifuge briefly once again.

7. Finger vortex all samples and briefly centrifuge. Let the samples incubate for 5 minutes at room temperature.

8. Proceed to the standard curve protocol.

Example spectrum Syber Green I dsDNA sample




## Standard Curve Protocol

1. Clean both sampling pedestals with 2 uL of nuclease free deionized water.

2. Open upper arm and firmly blot the two pedestals with a dry lab wipe. Make sure there are no traces of lint on the pedestals before continuing.

3. Open the operating software. Click on the Nucleic Acid Quantitation button and select the SyberGreen method.

4. Add 2 uL of assay buffer (no dye, no sample) to the lower pedestal. Lower the arm and click F3 or the Blank button. When the measurement is complete, lift the arm and use a dry laboratory wipe to blot the buffer from both the bottom and upper measurement surfaces. Use a fresh aliquot of buffer to verify a proper baseline.

5. Under Measurement type, click on the Standards tab. Highlight the Reference standard.

6. Mix the reference solution (assay buffer and dye, no sample) briefly and transfer 2 uL of the solution onto the lower pedestal. Lower the arm and click F1 or the Measure button. A pop up window will ask for confirmation of the units. (Recommended ng/mL or pg/uL)

7. Measure up to 5 replicates of the reference solution using a fresh 2 uL aliquot for each measurement.

8. Select Standard 1 to enter a value. Enter values for up to 7 standards.

9. Mix the standard solution briefly and transfer 2 uL onto the lower pedestal. Lower the arm and click F1 or the Measure button. Measure up to 5 replicates of each standard using a fresh 2 uL aliquot for each measurement.

10. Once the standard curve is completed, select the Standard Curve Type (Interpolation, Linear, 2° polynomial, 3° polynomial) that best fits the standards data set.

11. Click on the Sample tab under Measurement Type, and enter the unknown samples' respective ID information. If a dilution of the unknown sample was made, enter the dilution factor in the box below the sample ID window.

12. Add 2 ul of the sample and use the F1 key or click the Measure button to initiate the measurement cycle. Use a fresh aliquot of sample for each measurement.