Thermo SCIENTIFIC

PROTOCOL NanoDrop 3300

Sybr® Gold ssDNA Assay

Introduction

Sybr® Gold (Invitrogen) is one of the most sensitive nucleic acid dyes on the market. As an intercalating dye with an affinity for binding all nucleic acids Sybr® Gold is typically used as a gel stain. However using the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer, Sybr® Gold can be used to quantitate purified dsDNA, RNA, and ssDNA quickly and accurately. With few viable fluorescent assay options for specially quantitaing oligonucleotides, Sybr® Gold becomes an important dye to consider.

When paired with the ultra low volume capabilities of the NanoDrop 3300, Sybr® Gold provides an accurate rapid means of quantitating minute amounts of purified single stranded DNA sample. Sybr® Gold has demonstrated a linear range on the NanoDrop 3300 from 1000 ng/ml to 2.5 ng/ml, and has shown to be effective in accurately quantitating oligos down to 10 base pairs in length. Variation in signal may be observed between longer and shorter oligos, however the overall linear range of the assay holds. The excitation maxima for Sybr® Gold is 495 nm and the emission maxima is ~550 nm.

Assay Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 2 uL 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
- 1-10 ul sterile nuclease free low retention tips

Reagents:

- Sybr® Gold nucleic acid gel stain Sterile Nuclease free 1x TE buffer pH 8.0 (10mM tris, 1 nM EDTA)
- Control ssDNA

Sybr® Gold Assay Protocol

This protocol has been written for a volume of 10 uL per standard to allow for five 2 ul replicates. It is configured for a final dye dilution of 1:10,000 or 1X dye concentration from the original stock solution. Dye intensity may be lot specific and the final dye dilution may need to be changed at the discretion of the individual investigator.

1. Dilute the Sybr® Gold reagent 1:100 in 1X TE pH 8.0 (The 1:100 dilution may be stable for up to 24 hours if refrigerated and protected from light.)

   *To make a 1:100 dye dilution transfer 10 ul of 10,000X stock Sybr® Gold Dye into 990 ul 1x TE into a clean amber or foil covered 1.5 mL microfuge tube. Mix well by inversion.

2. Create a dilution series of ssDNA controls ranging from 5 ng/ml to 2,000 ng/ml in nuclease free tubes. (Note: best results will be seen if the length of the control oligo is similar in size to the unknown sample.)

Example Dilution Series

ssDNA (ng/ml)	ssDNA uL	1X TE uL	Total Volume uL	In Assay (ng/ml)
2000			183.3	1000
1000	83.3	83.3	166.5	500
500	66.5	66.5	133.0	250
100	33.0	132.0	165.0	50
50	65.0	65.0	130.0	25
10	30.0	120.0	150.0	5
5	50.0	50.0	100.0	2.5

In assay describes the final concentration of ssDNA present in the reaction

3. Prepare a final 1:5,000 working solution of Sybr® Gold reagent by making a 1:50 from the initial 1:100 dilution in a 1.5 mL amber or foiled nuclease free tube. (**Optimal working dilution may be lot specific).

   *Dilute 20 ul of 1:100 Sybr® Gold Reagent into 980 ul 1X TE in an amber tube for a final working solution of 1:5000.

Assay Protocol (cont)

4. Prepare a reference standard using 5 ul of 1X TE and 5 ul of Sybr® Gold working solution in a light protected tube. Vortex after combining the solutions. Centrifuge briefly.

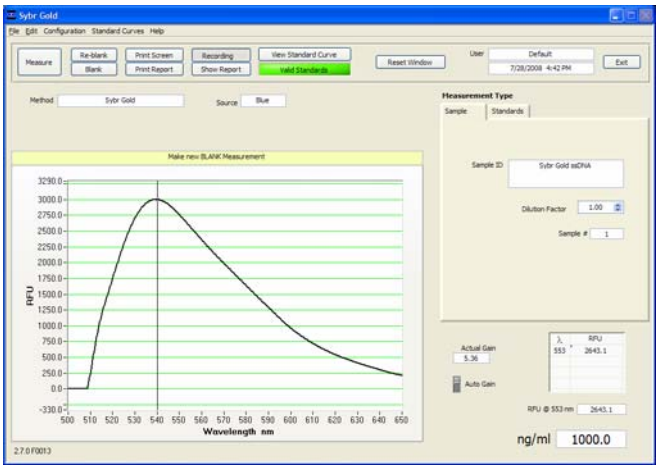
5. Add 5 ul of each ssDNA standard to a light protected tube. Centrifuge briefly and add 5 ul of Sybr® Gold working solution (1:1 from 1:5,000 working solution gives a 1x final Sybr® Gold concentration). Votex and centrifuge briefly.

6. Add 5 ul of each unknown ssDNA sample into a light protected tube and centrifuge briefly. Add 5 ul of working Sybr® Gold solution, centrifuge briefly.

7. Vortex or mix all samples to ensure homogeneity and briefly centrifuge. Allow the samples to incubate for 5 minutes at room temperature.

8. Proceed to standard curve protocol.

Example: Sybr® Gold Spectra



Standard Curve Protocol

1. Clean the sampling pedestals by adding 2 ul of nuclease free water to the bottom pedestal. Close the top pedestal and depress several times. Open the upper arm and firmly blot the two pedestals with a dry lab wipe. Ensure there are no traces of lint on the sampling pedestals before continuing.

2. Open the NanoDrop 3300 Operating software and select the Sybr® Gold Method

   ** If this is the first time you have measured Sybr® Gold you will need to set up a method. Select the Blue LED source. Click next and set the measurement wavelength to 550 nm. Proceed by clicking next. Name the method and set the graph min to 500 nm and the max to 600 nm.

3. Add 2 uL of 1X TE to the lower pedestal. Lower the arm and click the blank button (or press F3). At the completion of the blanking cycle raise the arm and firmly blot the upper and lower pedestals using a dry low lint laboratory wipe. Add a fresh aliquot of 1X TE and lower the pedestal. Click the measure button (or press F1) to ensure a proper baseline.

4. Under Measurement Type (In the upper right corner) select the Standards tab and click on Reference standard. Mix the reference solution, assay buffer and dye only, using the pipette tip and transfer 2 ul onto the lower pedestal. When you press the measure button a pop up will ask for confirmation of the units (recommended ng/ml or pg/ul). Measure up to 5 replicates of reference solution.

5. Select standard 1 and enter your lowest standard value. Mix standard 1 briefly and pipette 2 ul onto the lower pedestal. Press the measure button. Measure up to 5 replicates of each standard.

6. Repeat Step 5 for the remainder of the standards. Up to 7 standards can be measured.

7. After measuring the desired number of standards click the view standard curve button to ensure that the curve is linear.

8. Exit the standard curve window and click on sample under measurement type. Enter the unknown sample information under sample ID. Measure 2 ul aliquots of unknowns using a fresh aliquot for each sample measured.