Hoechst Dye 33258 Assay for dsDNA

Introduction

Hoechst 33258 dye is a fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA). Used in conjunction with the micro-volume capability of the Thermo Fisher NanoDrop™ 3300 Fluorospectrometer, the Hoechst 33258 dye provides 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. Hoechst 33258 dye circumvents such contributions from interfering substances by exhibiting an emission maximum at 450nm when bound specifically to dsDNA (unbound Hoechst 33258 dye 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 Hoechst 33258 dye of 75 ng/ml– 1500 ng/ml.

Hoechst Dye 33258 Assay Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 2uL pipettor (low retention nuclease free pipette tips)

Materials:

- Low lint laboratory wipes
- Hoechst Dye 33258 10mg/ml Solution (Molecular Probes catalog # R11496)
- 1X TNE buffer
- Nuclease free dH_2O

10X TNE Buffer Preparation

1. To a dH_2O rinsed 500 mL beaker add the following:
   - 70 mL of dH_2O
   - 20 mL 1M Tris Base
   - 10 mL 0.2M EDTA
   - 70 mL 5M NaCl

   Mix the solution thoroughly on a stir plate.

2. Use 1M HCL to titrate the 10X TNE buffer (pH of 7.4).

3. Transfer the contents of the beaker containing the 10X TNE solution to a graduated cylinder and adjust to a final volume of 200 ml.

4. Transfer the freshly prepared 10X TNE stock buffer to a clean 250 mL bottle. Store at 4°C and periodically check buffer for bacterial growth.

5. Dilute the 10X TNE ten (10) fold to obtain a 1X TNE working buffer solution by transferring 5mL of 10X TNE and 45 mL of dH_2O to a rinsed 250 mL beaker, mix well, and sterile filter for optimal performance. Store at 4°C and periodically check buffer for bacterial growth.

Hoechst Dye 33258 Assay Protocol

1. Equilibrate 1X TNE, Hoechst 33258 dye (10 mg/mL stock), standard dsDNA, and unknown dsDNA samples to room temperature. Mix each individual solution thoroughly.

2. Label 1.5 mL amber tubes with the appropriate standard concentrations (up to seven standards) and unknown sample tubes.

3. Prepare dsDNA standard dilutions and unknown samples at 2X the final concentrations in appropriate volumes for the assay. (Please refer to the example standard curve dilution series on page 2).

4. Dilute the Hoechst Dye stock (10 mg/mL) fifty-thousand (50,000) fold to obtain 0.2 ug/mL

   Dilute the Hoechst dye stock in two stages:

   a.Dilute the Hoechst stock (10 mg/ml) one-hundred (100) fold by transferring 495 uL of 1X TNE and 5uL of the 10mg/ml stock to a 1.5 mL snap cap tube and mix thoroughly. Protect from light.

   b. Dilute the primary Hoechst dye dilution five-hundred (500) fold to the working 2X solution by transferring 4.99 mL of 1X TNE and 10 uL of 0.1 mg/mL stock Hoechst dye to an 8mL amber screw top bottle and mix thoroughly. *Prepare fresh before each assay as lower dye concentrations will aggregate over time.*

5. Perform a 1:1 dilution by transferring an equal volume of the 0.2ug/ml Hoechst working solution to each 2X standard dsDNA solution and unknown sample (final concentration of the Hoechst dye will be 100ng/ml).

   Note: This assay may be scaled to fit the investigator's needs (i.e. add either 5 ul working solution and 5 ul of sample or 50 ul of working solution and 50 ul of sample.)

6. Prepare the Reference (negative control) solution by adding equal volumes of 1X TNE and 2X working Hoechst 33258 dye working solution.

7. Mix each assay dilution and sample thoroughly and allow to equilibrate on the bench top for five minutes.

8. Proceed to the standard curve protocol.

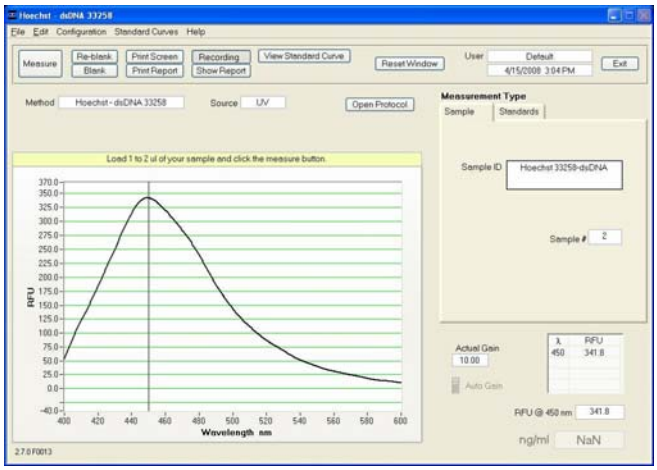
Example of standard curve dilution series.

Stock dsDNA conc. (ng/ml)	2X dsDNA volume (uL)	1X TNE buffer volume (uL)	2X dsDNA standard conc. (pg/uL)	Final dsDNA conc. (pg/uL)
3000	10*	0	3000	1500
3000	10**	10	1500	750
1500	10	10	750	375
750	10	10	375	188
375	10	10	188	94

** Initial stock undiluted* *** Initial stock diluted 1:1*

– The 2X dsDNA standard concentrations will be combined with the 2X Hoechst working solution to form the final 1X reaction mix.

Example spectrum for a Hoechst-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 Hoechst 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.