

Quant-iT™ dsDNA High Sensitivity (HS) Assay

Introduction

The Quant-iT™ dsDNA High Sensitivity Assays is a fluorescent nucleic acid stain for quantitating minute amounts of double-stranded DNA (dsDNA). Used in conjunction with the micro-volume Thermo Scientific NanoDrop™ 3300 Fluorospectrometer, the Quant-iT™ dsDNA High Sensitivity Assay provides a 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. Quant-iT™ dsDNA High Sensitivity reagent circumvents such contributions from interfering substances by exhibiting an emission maximum at ~530nm when bound to dsDNA (unbound Quant-iT™ dsDNA High Sensitivity reagent exhibits minimal fluorescence in solution). The ability of the NanoDrop 3300 allows significantly scaled-down reaction volumes, thereby using only a fraction of sample commonly needed for conventional cuvette-based fluorometers. Moreover, the Nanodrop 3300 has demonstrated a linear dynamic range for the Quant-iT™ dsDNA High Sensitivity assay of 1 ng/ml – 500 ng/ml. Measurements taken at the detection limit consume a total sample mass of 2 picograms of dsDNA.

Assay Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 2uL pipettor

Materials:

- Lab 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:

- Quant-iT™ dsDNA High Sensitivity (HS) kit (Invitrogen Cat# Q33120 or Q32851)
- Control dsDNA

Assay Protocol

1. Allow the Quant-iT™ dsDNA HS kit and unknown dsDNA samples to warm to room temperature and mix each component well.
2. Prepare the Quant-iT™ dsDNA HS working solution by transferring 5 uL of the 200X concentrated dye and 995 uL of the Quant-iT™ dsDNA HS buffer to a 1.5mL pre labeled amber snap cap tube and mix well.
3. Serially dilute the 100,000 ng/ml control dsDNA provided with the kit for the standard curve.

Example dilution series;

dsDNA [ng/ml]	dsDNA uL	1X TE uL	Total Volume uL	*In assay dsDNA [ng/ml]
10000.0			187.6	500.0
5000.0	87.6	87.6	175.2	250.0
2500.0	75.2	75.2	150.4	125.0
1000.0	50.4	75.6	126.0	50.0
200.0	26.0	104.0	130.0	10.0
40.0	30.0	120.0	150.0	2.0
20.0	50.0	50.0	100	1.0

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

4. Record the standard dsDNA concentrations on each tube and transfer 40uL of the Quant-iT DNA HS working reagent solution to each standard and unknown tube. Centrifuge the samples briefly to collect the dye at the bottom of the tube.
5. Add 2 uL of each standard and unknown dsDNA sample into the respective labeled nuclease free amber tube containing the dye working solution. Centrifuge the samples briefly to collect the dye at the bottom of the tube .
- 6 Prepare a reference standard (0 pg/uL dsDNA) by adding 40uL of the Quant-iT DNA HS working dye solution into a labeled nuclease free amber tube, centrifuge briefly, than and add 2uL of the Quant-iT DNA HS buffer centrifuge briefly once again
7. Finger vortex all samples and briefly centrifuge to collect the reactions at the bottom of the tubes. Let the samples and standards incubate for 5 minutes at room temperature.
8. Proceed to the standard curve protocol.

Standard Curve Protocol

1. Clean both sampling pedestals with 2 uL of nuclease free de-ionized 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 Quant-it DNA HS 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^o polynomial, 3^o 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.

Example spectrum Quant-it DNA HS sample:

