



A Modulus™ Method for DNA Quantitation Using Hoechst 33258

1. INTRODUCTION

Quantitation of DNA is an important step for many practices in molecular biology. Common techniques that use DNA, such as sequencing, cDNA synthesis and cloning, RNA transcription, transfection, nucleic acid labeling (e.g. random prime labeling), etc., all benefit from a defined template concentration. Failure to produce results from these techniques can sometimes be attributed to an incorrect estimate of the DNA template used. The concentration of a nucleic acid is most commonly measured by UV absorbance at 260 nm (A_{260}). Absorbance methods are limited in sensitivity, however, due to a high level of background interference.

Hoechst 33258, a bisbenzimidazole DNA intercalator, provides a fluorometric alternative that is more sensitive than UV absorbance methods. Hoechst 33258 excites in the near UV (350 nm) and emits in the blue region (450 nm). The sensitivity of the Modulus with Hoechst 33258 and dsDNA is better than 10 ng/mL (See Figure 1).

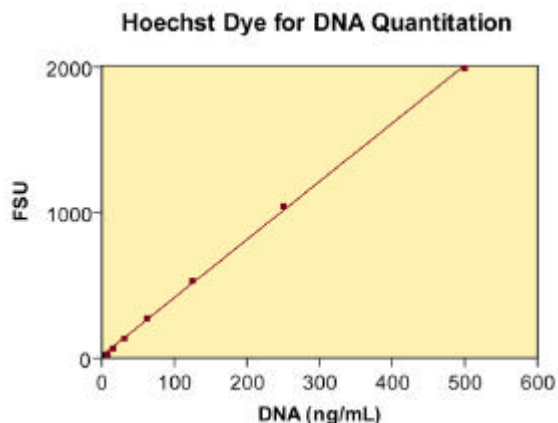


Figure 1. dsDNA and Hoechst dye analyzed on the Modulus and the UV Fluorescence Optical Kit. 1 μ g/mL of DNA was serially diluted in 1XTNE before the addition of 2XHoechst Dye working solution. After a 5-minute equilibration period, 100 μ L of each sample was transferred to a minicell cuvette and read in the Modulus fluorometer.

2. MATERIALS REQUIRED

- Modulus Fluorometer (P/N 9200-000 or 9200-002)
 - UV Fluorescence Optical Kit (P/N 9200-041)
 - Minicell cuvettes (P/N 7000-950) and minicell adaptor (P/N 9200-928)
- Or
- Methacrylate cuvettes (P/N 7000-959)
 - Hoechst 33258 10 mg/mL (Molecular Probes H3569)
 - 10XTNE buffer stock solution
 - 0.45 μ m filtered water

3. FACTORS TO CONSIDER

3.1 The AT content of a DNA sample affects Hoechst 33258-DNA fluorescence. Hence, it is important to use a standard similar to the samples you are testing. Calf Thymus DNA can often serve as a reference for most plant and animal DNA because it is double-stranded, highly polymerized, and is approximately 58% AT (42% GC). For bacterial DNA, a different standard may be needed because the AT content varies widely depending on the species.

3.2 The conformation (supercoiled, relaxed, circular, linear) of plasmid DNA may result in different Hoechst 33258 binding efficiencies. Thus, it is important to select a standard with similar physical characteristics to your sample.

3.3 Hoechst 33258 fluoresces only about half as much when it binds to single-stranded genomic DNA compared to when it binds to double-stranded genomic DNA. In addition, short pieces of single-stranded DNA will not normally cause Hoechst 33258 to fluoresce in proportion to their concentration.

3.4 Buffers commonly used to extract DNA from whole cells have little or no effect on this assay.

3.5 Low levels of detergent (<0.01% SDS) have little or no effect on this assay.

3.6 Salt concentrations in the sample extract of up to 3 M NaCl do not affect this assay. For peak fluorescence, at least 200 mM NaCl is required for purified DNA, and 2.0 to 3.0 M is required for crude samples. In crude samples, higher salt concentrations appear to cause the dissociation of proteins from DNA, allowing the dye molecules to bind to DNA easier.

3.7 RNA does not interfere significantly with the DNA assay because Hoechst 33258 does not normally bind to RNA. Under high salt concentrations, fluorescence from RNA is usually less than 1% of the signal produced from the same concentration of DNA.

4. REAGENT PREPARATION

NOTE: Hoechst 33258 is a possible carcinogen and possible mutagen. Wear gloves and a mask, and work under a fume hood.

4.1 Hoechst 33258 stock dye solution (1 mg/mL):
Dilute 1 mL Hoechst 33258 (10 mg/mL solution) with 9 mL distilled, 0.45 µm filtered water. Store in an amber bottle at 4°C for up to 6 months.

4.2 10X TNE buffer stock solution:
Dissolve into 800 mL of distilled water:
12.11 g Tris base [Tris (hydroxymethyl) aminomethane], MW = 121.14
3.72 g EDTA, disodium salt, dihydrate, MW = 372.20
116.89 g Sodium chloride, MW = 58.44
Adjust pH to 7.4 with concentrated HCl.
Add distilled water to 1000 mL.
Filter (0.45 µm) before use.
Store at 4°C for up to 3 months.

***NOTE:** The pH and NaCl concentration are essential for proper binding of the Hoechst reagent.

4.3 1XTNE: Dilute 10 mL 10XTNE with 90 mL distilled, 0.45 µm filtered water.

4.4 To prepare a 2X Dye Solution (200 ng/mL) for 10-1000 ng/mL final DNA concentration:
Dilute 20 µL Hoechst 33258 stock solution (1 mg/mL) with 100 mL 1XTNE. Keep assay

solution at room temperature. Prepare fresh daily. Do not filter once dye has been added.

4.5 Calf thymus DNA standard:
Prepare a 1 mg/mL stock solution of calf thymus DNA in 1XTE. Gently tap the tube to mix thoroughly. Store at 4°C for up to 3 months.

5. Instrument Set-Up

5.1 Power OFF the Modulus. Insert the UV Fluorescence Optical Kit according to the *Operating Manual*.

5.2 Power ON the Modulus and allow a 5-minute warm up period before calibration.

5.2 Protocol for 10 x 10 mm Standard Methacrylate Cuvettes

5.2.1 Prepare a 1-mL of 2000 ng/mL dsDNA standard solution for the calibration standard in a standard methacrylate cuvette.

NOTE: Polystyrene cuvettes are not compatible with the UV Fluorescence Optical Kit.

5.2.2 Add 2XHoescht dye working solution at a 1:1 ratio to the 2000 ng/mL DNA standard. The final concentration is 1000 ng/mL.

5.2.3 In a separate cuvette, prepare a blank solution by adding 2XHoescht dye working solution at a 1:1 ratio with 1XTNE buffer. The minimum volume is 2 mL.

5.2.4 Calibrate the Modulus with 1000 ng/mL.

NOTE: To optimize the accuracy, use a standard that is at or near the concentration of a typical sample. For example, if a typical sample is 300 ng/mL DNA, use a standard of 500 ng/mL DNA. The standard should be at or above 100 ng/mL.

5.2.5 Save the calibration for future use (optional)

5.2.6 Add 1 mL of 2XHoescht dye to 1 mL of a sample in a methacrylate cuvette. If necessary, dilute the samples with 1XTNE.

NOTE: The minimum volume is 2 mL in a 10 x 10 mm methacrylate cuvette.

5.2.7 Allow the samples and Hoechst dye a 5-minute equilibration period before reading in the Modulus.

5.2.8 The concentration of the sample and dye solution will appear on the Modulus screen.

NOTE: The final concentration is at least ½ of the original concentration due to the addition of the Hoechst dye. In addition, it is important to calculate for any dilutions of the original sample.

5.3 Protocol for Minicell Cuvettes

5.3.1 Prepare the standard solution. Dilute 1 mg/mL stock solution of DNA to a concentration of 2 µg/mL in 1XTNE. Add an equal volume of the 2XHoechst dye working solution, prepared in step 4.4. Mix well in a microcentrifuge tube.

NOTE: To optimize the accuracy, use a standard that is at or near the concentration of a typical sample. For example, if a typical sample is 300 ng/mL DNA, use a standard of 500 ng/mL DNA. The standard should be at or above 100 ng/mL.

5.3.2 Prepare the blank solution. Add an equal volume of the sample buffer (usually 1xTNE) to 2XHoechst dye working solution in a separate microcentrifuge tube.

5.3.3 Mix equal volumes of the sample with 2XHoechst dye working solution in a separate microcentrifuge tube.

NOTE: Do not mix samples, standard or blank solution with the Hoechst dye in the minicell cuvette.

5.3.4 Transfer 100 µL of the each sample, standard and blank solution to a minicell cuvette. Incubate for 2-5 minutes at room temperature, protected from light.

NOTE: Do not introduce air bubbles in the minicell cuvette. Air bubbles will cause erroneous readings.

5.3.5 Calibrate the Modulus with 1000 ng/mL.

5.3.6 Save the calibration for future use (optional).

5.3.7 Measure the sample solutions the concentration of DNA in the minicell cuvette will appear on the touchscreen.

NOTE: The final concentration is at least ½ of the original concentration due to the addition of the Hoechst dye. In addition, it is important to calculate for any dilutions of the original sample.

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