A GloMax®-Multi Jr Method for DNA Quantitation Using Hoechst 33258

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 sometimes can be attributed to an incorrect estimate of the DNA template used. The concentration of a nucleic acid most commonly is 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 bisbenzimide 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 GloMax®-Multi Jr with Hoechst 33258 and dsDNA is better than 10 ng/mL (see Figure 1).

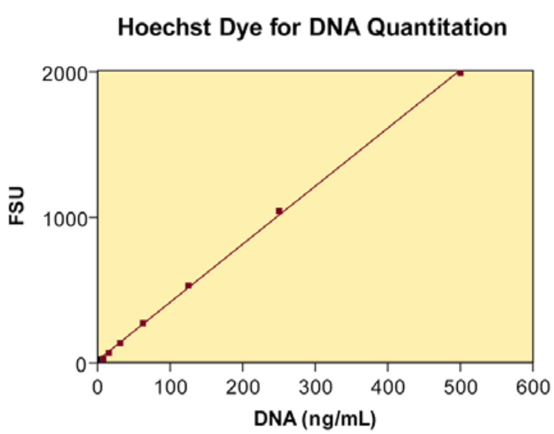

Figure 1. *dsDNA and Hoechst dye analyzed on the GloMax®-Multi Jr and the UV Fluorescence Optical Kit. One microgram per milliliter of DNA was serially diluted in 1X TNE before the addition of 2XHoechst Dye working solution. After a 5-minute equilibration period, 100 µL of each sample was transferred to a minicell cuvette and read in the GloMax®-Multi Jr.*

MATERIALS REQUIRED

- GloMax®-Multi Jr
- UV Fluorescence Optical Kit
- Minicell cuvettes and minicell adaptor or Methacrylate cuvettes
- Hoechst 33258 10 mg/mL
- 10X TNE buffer stock solution
- 0.45 µm filtered water

FACTORS TO CONSIDER

- 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 often can serve as a reference for most plant and animal DNA because it is double-stranded, highly polymerized and approximately 58% AT (42% GC). For bacterial DNA, a different standard may be needed because the AT content varies widely depending on the species.
- 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.
- 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 normally will not cause Hoechst 33258 to fluoresce in proportion to their concentration.
- Buffers commonly used to extract DNA from whole cells have little or no effect on this assay.
- Low levels of detergent (<0.01% SDS) have little or no effect on this assay.
- 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.

- 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.

REAGENT PREPARATION

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

- 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.
- 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.
- 1X TNE: Dilute 10 mL 10X TNE with 90 mL distilled, 0.45 µm filtered water.
- 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 1X TNE. Keep assay solution at room temperature. Prepare fresh daily. Do not filter once dye has been added.
- Calf thymus DNA standard: Prepare a 1 mg/mL stock solution of calf thymus DNA in 1X TE. Gently tap the tube to mix thoroughly. Store at 4°C for up to 3 months.

INSTRUMENT SETUP

- Power OFF the GloMax®-Multi Jr. Insert the UV Fluorescence Optical Kit according to the Technical Manual.
- Power ON the GloMax®-Multi Jr, and allow a 5-minute warm-up period before calibration.

PROTOCOL FOR 10 x 10 mm STANDARD METHACRYLATE CUVETTES

- Prepare 1 mL of a 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.
- Add 2X Hoescht dye working solution at a 1:1 ratio to the 2000 ng/mL DNA standard. The final concentration is 1000 ng/mL.
- In a separate cuvette, prepare a blank solution by adding 2X Hoescht dye working solution at a 1:1 ratio with 1X TNE buffer. The minimum volume is 2 mL.
- Calibrate the GloMax®-Multi Jr 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.
- Save the calibration for future use (optional).
- Add 1 mL of 2X Hoecsht dye to 1 mL of a sample in a methacrylate cuvette. If necessary, dilute the samples with 1X TNE.
  Note: The minimum volume is 2 mL in a 10 x 10 mm methacrylate cuvette.
- Allow the samples and Hoechst dye a 5-minute equilibration period before reading in the GloMax®-Multi Jr.
- The concentration of the sample and dye solution will appear on the GloMax®-Multi Jr 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.

PROTOCOL FOR MINICELL CUVETTES

- 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 2X Hoechst dye working solution, prepared in Reagent Preparation section. 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.

- Prepare the blank solution. Add an equal volume of the sample buffer (usually 1X TNE) to 2X Hoechst dye working solution in a separate microcentrifuge tube.
- Mix equal volumes of the sample with 2X Hoechst 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.
- Transfer 100 µL of 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.
- Calibrate the GloMax®-Multi Jr with 1000 ng/mL.
- Save the calibration for future use (optional).
- Measure the sample solutions. The concentration of DNA in the minicell cuvette will appear on the touch 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.

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