

FluoReporter® Blue Fluorometric dsDNA Quantitation Kit (F-2962)

Quick Facts

Storage upon receipt:

- 2–6°C
- Protect from light

Abs/Em: (bound to DNA) 346/460 nm

Number of assays: 2000 microplate assays

Introduction

The bisbenzimidazole derivative Hoechst 33258 exhibits fluorescence enhancement upon binding to A-T rich regions of double stranded DNA.^{1,2} DNA binding specificity is enhanced under high ionic strength conditions. Several fluorometric methods for analysis of DNA in solutions and cells have been developed by exploiting these properties.³⁻⁵ This kit implements the microplate procedure devised by Rago et al.⁵ The procedure is rapid, requires no extraction reagents for exposure of DNA to the fluorescent

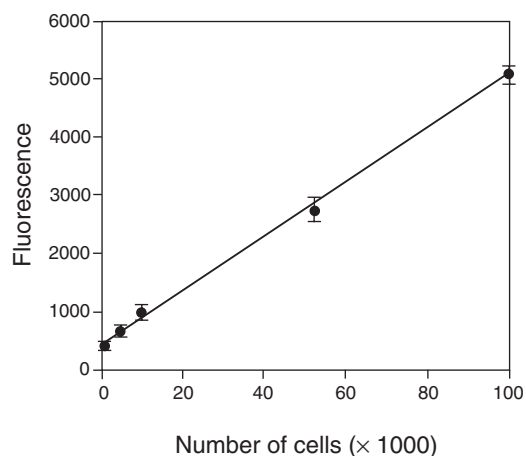


Figure 1. Fluorometric analysis of 3T3 cells according to standard protocol described in Experimental Protocol. Fluorescence measurements were carried out using excitation at 360 nm and detection at 460 nm.

probe (this is accomplished by freezing in distilled water to cause cell lysis), and all manipulations are carried out in the microplate wells. The assay can be used to count adherent cells in the range of approximately 1000 to 100,000 per well on the basis of DNA content (Figure 1) for proliferation studies, or alternatively for DNA quantification by comparison with a standard curve.

Preparation

Contents and Storage

- **Hoechst 33258** (Component A), concentrated solution in DMSO/H₂O 1:4 (v/v), 0.5 mL
- **TNE buffer** (Component B), 10 mM Tris, 2 M NaCl, 1 mM EDTA, pH 7.4, containing 2 mM sodium azide, 200 mL

The contents are sufficient for 2000 microplate assays, following the standard protocol (see *Experimental Protocol*). These reagents should be stored in a refrigerator until required.

Reagent Preparation

Prepare aqueous dye reagent by adding 25 μ L of the Hoechst 33258 solution provided to 10.0 mL of TNE buffer. This is sufficient for 100 samples ($\sim 1 \times 96$ well microplate) according to the standard protocol. This reagent may be stored in a refrigerator if not immediately required.

Materials Required But Not Provided

- Microplates with a capacity of ≥ 250 μ L per well
- Calf thymus DNA and TE buffer for dilution (only if a standard curve is required)

DNA Standard Curve (Optional)

1.1 Prepare a 10 μ g/mL stock solution of calf thymus DNA (for example Sigma Chemical Co., catalog number D-1501) in TE buffer (10 mM Tris base, 1 mM EDTA, adjust to pH 7.4 with concentrated HCl). Determine DNA concentration on the basis of absorbance at 260 nm in a 1 cm path length cuvette ($A_{260\text{ nm}} = 0.2$ for 10 μ g/mL). 300 μ L of this stock solution is sufficient for a single replicate 9-point standard curve (step 1.2). Prepare 200 μ L of 10-fold diluted (1 μ g/mL) stock solution.

1.2 Dilute the standards into a row of microplate wells as follows:

μL of 10 $\mu\text{g}/\text{mL}$ DNA	μL of 1 $\mu\text{g}/\text{mL}$ DNA	μL of TE buffer	DNA (ng/100 μL)
100	0	0	1000
75	0	25	750
50	0	50	500
25	0	75	250
10	0	90	100
0	75	25	75
0	50	50	50
0	25	75	25
0	10	90	10
0	0	100	0

1.3 Follow steps 2.8 to 2.10 of experimental protocol.

1.4 Calculate the fluorescence intensity per ng of DNA (~slope of linear calibration plot). This calibration compared with a calibration of fluorescence intensity for known cell numbers under the same dye concentration and detection sensitivity conditions can be used to estimate the amount of DNA per cell:

$$\frac{\text{fluorescence intensity per cell}}{\text{fluorescence intensity per ng of DNA}} = \text{ng DNA per cell}$$

Using a calibration range of 5 to 500 ng of calf thymus DNA, we obtained a value of 13.3 pg DNA per 3T3 cell (data from Figure 1).⁶ This is in excellent agreement with published values.⁷

Experimental Protocol

2.1 Transfer adherent cells of interest into a concentrated cell stock (~10⁶ cells/mL) in normal medium.

2.2 Prepare serial dilutions of cells in microplate wells, resulting in 100 μL volumes each, containing between 1000 and 100,000 cells per well (note A).

2.3 Incubate the plate at 37°C for the time required for the cells to attach (between 4 and 16 hours) or to proliferate.

2.4 Empty wells at desired endpoint by overturning onto paper towels; store at -80°C until ready to scan (note B).

2.5 Thaw plates to room temperature and add 100 μL distilled water per well.

2.6 Incubate at 37°C for 1 hour.

2.7 Place plate at -80°C until frozen, then thaw to room temperature (note C).

2.8 Add 100 μL of aqueous Hoechst 33258 in TNE buffer (prepared in *Reagent Preparation*; note D).

2.9 Measure fluorescence using excitation and emission filters centered at 360 nm and 460 nm, respectively. Alternative filters compatible with the excitation and emission spectra of Hoechst 33258 bound to DNA may also be used (the fluorescence excitation and emission maxima are 346 nm and 460 nm, respectively).

2.10 Subtract the reagent blank fluorescence values (100 μL of aqueous dye (as described in *Reagent Preparation*) + 100 μL distilled water) from the sample data before plotting or other subsequent analysis.

Notes

[A] When first performing the assay, it may be desirable to prepare a standard curve of a known cell number using a hemacytometer.

[B] To reduce the standard error, plates may be stored for up to 4 weeks at -80°C and those from different time points can be scanned sequentially.

[C] This procedure causes rapid cell lysis, resulting in release of DNA to form a relatively homogenous solution.

[D] With large numbers of cells (greater than 100,000), improved analytical linearity may be obtained by increasing the final concentration of Hoechst 33258 (use 50 μL of the DMSO/H₂O stock solution instead of the 25 μL in as described in *Reagent Preparation*).

References

1. J Histochem Cytochem 24, 24 (1976); 2. Biochemistry 29, 9029 (1990); 3. Anal Biochem 102, 344 (1980); 4. Anal Biochem 131, 538 (1983); 5. Anal Biochem 191, 31 (1990); 6. E.M. Marcus and I.D. Johnson, unpublished results; 7. Methods Enzymol 58, 141 (1979).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
F-2962	FluoReporter® Blue Fluorometric dsDNA Quantitation Kit *200-2000 assays*	1 kit

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