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# Product Information

**DNA Quantitation Kit, Fluorescence Assay**

Catalog Number **DNAQF**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Fluorometry is a highly sensitive and simple method for DNA quantitation. The fluorescent dye, bisBenzimide H 33258 (Hoechst 33258), which binds primarily to AT sequences in the minor groove of double-stranded DNA (dsDNA), is specific for quantitation of nanogram amounts of DNA (10 ng/ml to 10 μg/ml).[1-3,6] When excited at 360 nm, the fluorescence emission at 460 nm of the dye increases significantly in the presence of DNA.

bisBenzimide H 33258 works well with purified preparations of DNA as well as with DNA from crude extracts that may be contaminated with RNA and proteins.[1,4,6] However, the quantitation of DNA in crude extracts has been reported to require a high salt concentration.[1]

Linear and circular dsDNA will give similar fluorescence intensities, but the binding efficiency of the dye to single-stranded DNA (ssDNA) is lower than the binding efficiency to dsDNA.[5] RNA can also enhance fluorescence, but to a much lesser extent than dsDNA and ssDNA. This enhancement is reduced in the presence of high salt concentration.[1]

The minimum length of dsDNA required for detection depends on the DNA sequence. For most applications, dsDNA of at least 200 bp in length can be detected by the bisBenzimide assay. Shorter molecules can be detected provided two or three consecutive AT bp are present.[3] The assay is not suitable for use with single stranded oligomers.

### Components

The kit is sufficient for 750 reactions (50 experiments of 15 reactions each, 2 ml volume)

- DNA Standard 1 ml
  Catalog Number D4810
  1 mg/ml solution of calf thymus DNA
  in 10 mM Tris HCl, pH 7.4, with 1 mM EDTA
- bisBenzimide H 33258 Solution 250 μl
  Catalog Number B1302
  10 mg/ml bisBenzimide H 33258 (Hoechst 33258)
  in deionized water
- 10× Fluorescent Assay Buffer 150 ml
  Catalog Number F7171
  100 mM Tris HCl, pH 7.4, with 10 mM EDTA
  and 2 M NaCl

### Equipment and Reagents Required but Not Provided

- Multiwell plates
- Fluorometer
- Molecular Biology Grade Water (Catalog Number W4502)

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

1. 10× Fluorescent Assay Buffer (Catalog Number F7171) - After the initial thaw, mix until the solution is completely homogeneous and store at 2–8 °C.

2. 100 μg/ml and 10 μg/ml DNA Stock Solutions - Thaw the DNA Standard (Catalog Number D4810, 1 mg/ml) and incubate at 50 °C for 15–30 minutes. Store an aliquot at 2–8 °C. The remaining DNA Standard should be stored at –20 °C. More than 4 freeze/thaw cycles are **not** recommended for the DNA Standard. After each thawing, the DNA Standard should be incubated at 50 °C for 15–30 minutes to ensure the material is completely dissolved. Dilute the DNA Standard to two concentrations, 100 μg/ml and 10 μg/ml of DNA (see Table 1), using sterile pipettes and tubes. Mix and store the prepared solutions at 2–8 °C for up to 6 months.

**Table 1.**
Preparation of DNA Stock Solutions

| | DNA Stock Solution (100 µg/ml) | DNA Stock Solution (10 µg/ml) |
|---|---|---|
| DNA Standard, calf thymus DNA (1 mg/ml) | 100 µl | 10 µl |
| 10× Fluorescent Assay Buffer | 100 µl | 100 µl |
| Molecular Biology Grade Water | 800 µl | 890 µl |
| Total Volume | 1 ml | 1 ml |

Note: This kit includes calf thymus DNA for preparation of a DNA standard curve. In most cases the calf thymus DNA, which has 58% AT content, is suitable as a standard DNA. For the assay of DNA with a significantly different base composition, a DNA standard with a similar base composition should be used.

3. bisBenzimide H 33258 Solutions - Dilute an aliquot of the 10 mg/ml bisBenzimide H 33258 (Catalog Number B1302) 10-fold with molecular biology grade water to a concentration of 1 mg/ml. Store this solution in the dark at 2–8 °C. The 1 mg/ml bisBenzimide H 33258 Solution can be stored up to 6 months. Prepare dye solutions for generation of the standard curve and measurement of unknown DNA concentrations from the 1 mg/ml dye solution (see Table 2). Add the appropriate amounts to a tube and mix. These solutions should be made fresh just prior to use and stored in the dark.

**Table 2.**
Preparation of bisBenzimide H 33258 Solutions

| | bisBenzimide H 33258 Solution 1 µg/ml | bisBenzimide H 33258 Solution 0.1 µg/ml |
|---|---|---|
| bisBenzimide H 33258 Solution (1 mg/ml) | 30 µl | 3 µl |
| 10× Fluorescent Assay Buffer | 3 ml | 3 ml |
| Molecular Biology Grade Water | 27 ml | 27 ml |
| Total Volume | 30 ml | 30 ml |

Both of the bisBenzimide H 33258 Solutions (30 ml) are sufficient for 15 determinations, using 2 ml per sample (7 levels for the standard curve and 8 samples to be measured).

**Storage/Stability**
Store the kit at –20 °C.

**Procedure**
The bisBenzimide H 33258 assay requires a DNA standard calibration curve to determine the DNA content of an unknown sample. A dye solution with concentration of 0.1 µg/ml is adequate for the analysis of DNA up to ~500 ng. A dye concentration of 1 µg/ml will extend the assay's range up to 10 µg, but will limit the sensitivity at low concentrations. For accurate determination of the DNA concentration, two standard curves are suggested, see Tables 3 and 4. Low concentrations of DNA may give low relative fluorescence unit values that are within the linear range.

This kit may be used to determine the DNA content of biological samples. The detergents, SDS and IGEPAL® CA-630, have no effect on the assay at final concentrations of 0.001% and 0.0001%, respectively. In addition, $Mg^{2+}$ ions have no effect on the assay in the final concentration range of 0.5 mM to 0.1 M. The fluorescence of the bisBenzimide H 33258-DNA complex is optimal at pH 7.4. With the pH less than 6.0 or greater than 8.5, the fluorescence is very weak.

1. Turn on the fluorometer and allow it to warm up. Set the excitation wavelength to 360 nm and the emission wavelength to 460 nm.

2. Prepare 0.1 µg/ml and 1 µg/ml bisBenzimide H 33258 Solutions in 10× Fluorescent Assay buffer (see Preparation Instructions, Step 3) and store in the dark until ready to use. Prepare sufficient amounts of the dye solutions for two standard curves as shown in Tables 3 and 4, and for the sample determinations. The DNA Stock Solutions will be added directly to the cuvette in the fluorometer.

**Table 3.**
Reaction scheme for 0.1 μg/ml bisBenzimide H 33258 Solution with DNA in the range of 10–500 ng/ml

| Sample | DNA Stock Solution (10 μg/ml) | DNA Stock Solution (100 μg/ml) | bisBenzimide H 33258 Solution (0.1 μg/ml) | Final Amount of DNA in 2 ml Reaction |
|---|---|---|---|---|
| 1 | – | – | 2 ml | Blank |
| 2 | 2 μl | – | 2 ml | 20 ng* |
| 3 | 5 μl | – | 2 ml | 50 ng |
| 4 | 10 μl | – | 2 ml | 100 ng |
| 5 | – | 2 μl | 2 ml | 200 ng |
| 6 | – | 5 μl | 2 ml | 500 ng |
| 7 | – | 10 μl | 2 ml | 1,000 ng |

*An additional standard of 10 ng/2 ml may be detected using some fluorometers.

**Table 4.**
Reaction scheme for 1 μg/ml bisBenzimide H 33258 Solution with DNA in the range of 100 ng to 5 μg/ml

| Sample | DNA Stock Solution (100 μg/ml) | DNA Standard (1 mg/ml) | bisBenzimide H 33258 Solution (1 μg/ml) | Final Amount of DNA in 2 ml Reaction |
|---|---|---|---|---|
| 1 | – | – | 2 ml | Blank |
| 2 | 2 μl | – | 2 ml | 200 ng |
| 3 | 5 μl | – | 2 ml | 500 ng |
| 4 | 10 μl | – | 2 ml | 1 μg |
| 5 | – | 2 μl | 2 ml | 2 μg |
| 6 | – | 5 μl | 2 ml | 5 μg |
| 7 | – | 10 μl | 2 ml | 10 μg * |

*An additional standard of 20 μg/2 ml may be detected using some fluorometers.
Note: The ability to read DNA standard points at the upper and lower limits of the standard curve may be dependent on the type of fluorometer being used.

3. Pipette 2 ml of the appropriate dye solution (prepared in step 2) into the cuvette and place in sample chamber.

4. Read the blank at 360 nm excitation and 460 nm emission at ambient temperature. Using slits set at 2.5 nm for the 0.1–5 μg/ml range and 5 nm for the 10–500 ng/ml range may increase the range of DNA detected.

5. With the cuvette still in the fluorometer, add the appropriate DNA solution according to Table 3 or 4 to the cuvette. Mix the solutions in the cuvette and read the emission. This will reduce photobleaching of the reaction mixture.
   Note: The volume of the DNA solution added to the dye solution should not exceed 10 μl.

6. Wash the cuvette and repeat steps 4 and 5 with the remaining DNA standards and the unknown samples using fresh dye solutions.

Note: Multiwell plate assay - DNA quantitation using the bisBenzimide H 33258 assay may be performed using a multiwell plate in a suitable fluorometer. The sensitivity of plate readers varies between different fluorometers. It is recommended to use 200 μl of 2 μg/ml bisBenzimide H 33258 Solution. The range of DNA concentrations to be measured should be between 0.1–10 ng/μl

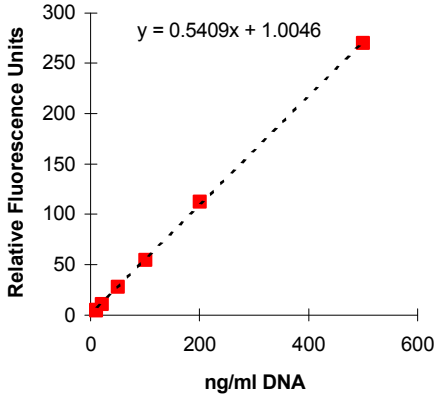
Calculations

1. Prepare a calibration curve by plotting total DNA concentration versus relative fluorescence units, see Figure 1.

2. Determine the least squares regression equation for the line generated by the standard samples. The linear equation is **y = mx + b**, where:

   **y** - Emission expressed in Relative Fluorescence Units (RFU)
   **m** - The slope
   **x** - DNA concentration
   **b** - The intercept

**Figure 1.**
An Example of a Standard Curve




The DNA range is 10–500 ng/ml

**References**

1. Labarca, C., and Paigen, K., Anal. Biochem., **102**, 344 (1980).
2. Portugal, J., and Waring, M.J., Biochem. Biophys. Acta, **949**, 158 (1988).
3. Teare, J.M., *et al*., Biotechniques, **22**, 1170 (1997).
4. Moe, D., *et al*., J. Biochem. Biophys. Methods, **28**, 263 (1994).
5. Daxhelet, G.A., *et al.*, Anal. Biochem., **179**, 401 (1989).
6. Cesarone, C.F., et al., Anal. Biochem.,**100**, 188 (1979).

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