Fluorescent DNA Quantitation Kit

Code	Description	Size
1B1558-KIT	**Fluorescent DNA Quantitation Kit**	750 x 2 mL reactions
1B1558-KIT-SAMPLE	**Fluorescent DNA Quantitation Kit**	75 x 2 mL reactions

General Information

VWR Life Science AMRESCO's Fluorescent DNA Quantitation Kit uses the fluorescent bisBenzimide H33258 dye to bind to AT sequences of the minor groove of double-stranded DNA. In the assay, the sample is excited at 360 nm and when the dye binds to dsDNA an emission spectrum at 460 nm is produced. AMRESCO's Fluorescent DNA Quantitation Kit can be used to quantify dsDNA in the low nanogram per mL (10 ng/mL) range, up to 5 microgram per mL (5 µg/mL). This assay can also tolerate RNA or protein contamination that may be present in crude extracts.

- Highly specific quantitation of DNA
- Sensitive to low nanogram per mL levels of DNA
- Assay tolerates RNA and protein contaminants

Storage/Stability

Fluorescent DNA Standard and **Fluorescent DNA Dye** should be stored frozen (-20 – 0 °C) and is stable for up to 1 year. Freeze-thaw cycles should be avoided. Fluorescent DNA Standard and Fluorescent DNA Dye Solution are stable several weeks cold (2 – 8 °C).

Fluorescent DNA Assay Buffer, 10X is stable for up to 2 years at room temperature (18 – 26°C).

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Materials Supplied

1B1558-KIT (sufficient for 750 x 2 mL reactions)
Fluorescent DNA Standard: 1 mL of a 1 mg/mL solution of calf thymus DNA in 10 mM Tris-HCl pH 7.4, 1 mM EDTA
Fluorescent DNA Dye: 250 µL of a 10 mg/mL bisBenzimide H 33258 solution in deionized water
Fluorescent DNA Assay Buffer, 10X: 200 mL of 100 mM Tris-HCl, pH 7.4, 2 M NaCl, 10 mM EDTA

1B1558-KIT-SAMPLE (sufficient for 75 x 2mL reactions)
Fluorescent DNA Standard: 0.1 mL of a 1 mg/mL solution of calf thymus DNA in 10 mM Tris-HCl pH 7.4, 1 mM EDTA
Fluorescent DNA Dye: 25 µL of a 10 mg/mL bisBenzimide H 33258 solution in deionized water
Fluorescent DNA Assay Buffer, 10X: 20 mL of 100 mM Tris-HCl, pH 7.4, 2 M NaCl, 10 mM EDTA

Required Materials Not Supplied

Cuvettes or multiwall plates
Fluorometer
Deionized water

Protocol/Procedure

Preparation of DNA: Thaw 10X Assay Buffer at room temperature

To prepare DNA Standard Solutions of 10 µg/mL and 100 µg/mL; You will use the 10 µg/mL and 100 µg/mL stock for the low range samples and the 100 µg/mL and 1 mg/mL stock for the high range samples.

1. Thaw the Fluorescent DNA Standard (1 mg/mL). If frozen, incubate at 50°C for 5 – 10 minutes to ensure that all DNA is solubilized. An aliquot stored at 2 – 8°C does not need to be heated.
2. Dilute the Fluorescent DNA standard using sterile pipettes and tubes. Use the table below to prepare the following 2 stock solutions:

Component	10 µg/mL DNA Stock	100 µg/mL DNA Stock
Fluorescent DNA Standard (1 mg/mL)	10 µL	10 µL
Fluorescent DNA Assay Buffer, 10X	100 µL	100 µL
Deionized Water	890 µL	800 µL
Total Volume	**1 mL**	**1 mL**

3. Store the Fluorescent DNA Standard (1 mg/mL) at -20°C. Do not free-thaw the samples more than 5 times. An aliquot of the 1 mg/mL DNA standard and the 10 µg/mL and 100 µg/mL DNA Stock Solutions may be stored at 2 – 8°C for several weeks.

Preparation of Dye:

To prepare bisBenzimide H 33258 Solutions of 0.1 µg/mL and 1.0 µg/mL; The 0.1 µg/mL bisBenzimide stock will be used for the low range samples and the 1.0 µg/mL bisBenzimide stock for the high range samples:

1. Dilute the Fluorescent DNA Dye to 1 mg/mL (ten-fold dilution) with deionized water. Solution should be stored protected from light at 2 – 8°C for several weeks.
2. Dilute the 1 mg/mL Fluorescent DNA Dye using sterile pipettes and tubes. Use Table 1 below to prepare the following 2 concentrations. Make these solutions just prior to use and store protected from light.

Component	0.1 µg/mL Fluorescent DNA Dye	100 µg/mL Fluorescent DNA Dye
Fluorescent DNA Assay Buffer, 10X	3 mL	3 mL
Deionized Water	27 mL	27 mL
Fluorescent DNA Dye (1 mg/mL)	3 µL	30 µL
Total Volume	**30 mL**	**30 mL**

Table 1

The amount of solution prepared in the table (30 mL) is sufficient to perform 15 assays (2 mL per assay). This is enough material to measure the fluorescence of one standard curve (seven samples) and 8 unknown samples.

Standard Curve and Sample Determination

A standard calibration curve must be performed to determine the DNA concentration of an unknown sample. Use the 0.1 µg/mL Fluorescent DNA Dye for DNA concentrations up to 500 ng/mL of DNA and the 1.0 µg/mL Fluorescent DNA Dye for higher DNA concentrations, up to 5 ug/mL of DNA.

1. Warm up the fluorometer for 15 – 20 minutes prior to use. Set the excitation wavelength at 360nm and the emission wavelength at 460nm.
2. Use the previously prepared Fluorescent DNA Dye solutions to create the standard curve samples. Label sterile tubes according to the chart below. Tubes should be able to hold 2 mL or more. Add additional tubes to account for the unknown samples that will be measured. Reference the Table 2 or Table 3, for steps 3 and 4 below, depending on the expected concentration range of the unknown samples.
3. Add 2 mL of the appropriate Fluorescent DNA Dye solution (determined from the expected concentration range of DNA) to each tube.
4. Add the appropriate volume of the proper DNA stock to each tube as referenced in the chart below. (Do not exceed 10 µL for the amount of DNA sample to add to the tube.) Mix the samples by vortexing.

DNA Concentration Range 10 – 500 ng/mL				
Sample	0.1ug/mL Fluorescent DNA Dye (mL)	10ug/mL DNA Stock (uL)	100ug/mL DNA Stock (uL)	Final DNA Concentration (ng/mL)
1	2	0	-	0
2	2	2	-	10
3	2	5	-	25
4	2	10	-	50
5	2	-	2	100
6	2	-	5	250

Table 2

DNA Concentration Range 10 – 500 ng/mL				
Sample	0.1ug/mL Fluorescent DNA Dye (mL)	10ug/mL DNA Stock (uL)	100ug/mL DNA Stock (uL)	Final DNA Concentration (ng/mL)
1	2	0	-	0
2	2	2	-	100
3	2	5	-	250
4	2	10	-	500
5	2	-	2	1000
6	2	-	5	2500
7	2	-	10	5000

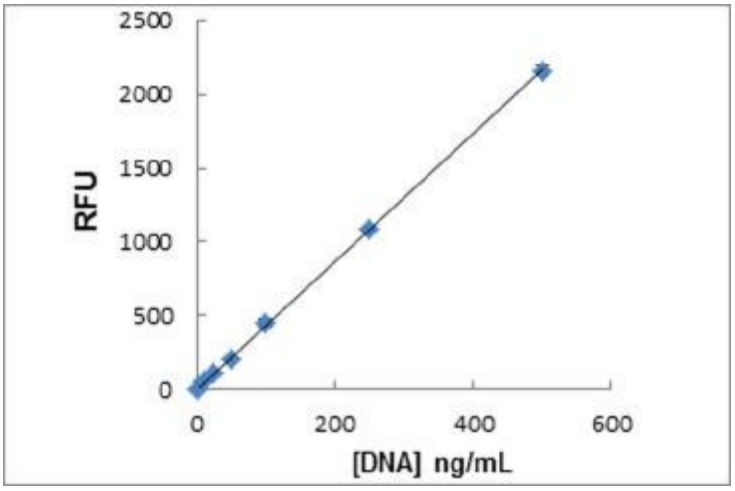
Table 3

5. Make sure that the excitation wavelength is 360 nm and the emission wavelength is 460 nm. Add sample 1, that does not contain DNA, to the cuvette and blank.
6. Wash the cuvette and add Sample 2, which contains DNA, to the cuvette. Monitor the fluorescence reading.
7. Repeat Step 7 until all of the samples have been monitored.
8. Prepare a standard curve using the seven known DNA samples by plotting DNA concentration versus relative fluorescence units (RFU). See image below.
9. Determine the equation of the line using least squares regression. This equation can be used to determine the concentration of the unknown samples by inputting the RFU of the unknown sample and solving for x.

Standard Curve Example

y = 4.3149x + 6.3684, where y=RFU, and x=DNA concentration

R^2 = 0.9999



For Technical Support

Toll Free: 1-800-610-2789 (USA & Canada)
Fax: (440) 349-0235
Email: techinquiry@amresco-inc.com

AMRESCO, LLC
A VWR Company

Corporate Headquarters
28600 Fountain Parkway
Solon, Ohio USA 44139-4300

Tel: 440/349-1199
Fax: 440/349-1182
www.amresco-inc.com

Fluorescent DNA Quantitation Kit

ZY0614
Rev. 1 12/2015