Fluorescent DNA Quantitation Kit

CODE	DESCRIPTION	SIZE
1B1558-KIT-SAMPLE	**Fluorescent DNA Quantitation Kit** Includes: • Fluorescent DNA Assay Buffer, 10X, 20 mL • Fluorescent DNA Dye, 25 µL • Fluorescent DNA Standard, 0.1 mL • Sufficient material for 75-2 mL reactions.	**100 Reactions**
1B1558-KIT	**Fluorescent DNA Quantitation Kit** Includes: • Fluorescent DNA Assay Buffer, 10X, 200 mL • Fluorescent DNA Dye, 250 µL • Fluorescent DNA Standard, 1 mL • Sufficient material for 750-2 mL reactions.	**1000 Reactions**

General Information

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.

Storage/Stability

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

Fluorescent DNA Assay Buffer, 10X is stable for up to 2 years at room temperature (18-25°C).

Product Use Limitations

For Research Use Only. Not for Therapeutic or Diagnostic Use.

Hazard Precaution

Please see the Safety Data Sheet for information regarding hazards and safe handling practices.

Supplied Materials

1B1558-KIT

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

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

Additional required materials not supplied

- Cuvettes or multiwell plates
- Fluorometer
- Deionized water

Protocol/Procedure

Initial Preparation:

- Before use, thaw the Fluorescent DNA Assay Buffer, 10X at room temperature.

Preparation of DNA:

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 1mg/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 concentrations:
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.

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**

2. Dilute the 1 mg/mL Fluorescent DNA Dye using sterile pipettes and tubes. Use the table below to prepare the following 2 concentrations:
3. Make these solutions just prior to use and store protected from light.

Component	0.1 µg/mL Fluorescent DNA Dye	1.0 µg/mL Fluorescent DNA Dye
Fluorescent DNA Assay Buffer, 10X	3 mL	3 mL
Deionized Water	27 mL	27 mL
Fluorescent DNA Dye (1mg/mL)	3 µL	30 µL
Total Volume	**30 mL**	**30 mL**

4. 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 Fluorescent DNA Dye (see initial preparation stage) to create the following samples:
3. Label seven sterile tubes, 1-7. Tubes should be able to hold 2 mL or more. Add additional tubes to account for the unknown samples that will be measured.
4. Add 2 mL of the proper bisBenzimide H 33258 dye solution (determined from the expected concentration range of DNA) to each tube.
5. Add the appropriate DNA solution and amount to each tube. (Do not exceed 10 µL for the amount of DNA sample to add to the tube.) Mix the samples by vortexing.
6. Add the first sample, that does not contain DNA, to the cuvette and blank. Make sure that the excitation wavelength is 360nm and the emission wavelength is 460nm.
7. Wash the cuvette and add Sample 2, which contains DNA, to the cuvette. Monitor the fluorescence reading.

DNA Concentration Range: 10-500ng/mL				
Sample	0.1 µg/mL Fluorescent DNA Dye (mL)	10 µg/mL DNA Stock (µL)	100 µg/mL DNA Stock (µL)	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

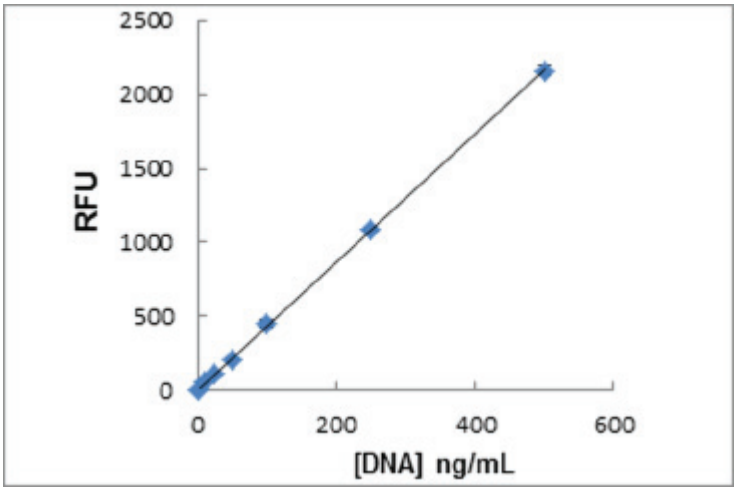
DNA Concentration Range: 100-5,000ng/mL (100ng/mL – 5ug/mL)				
Sample	1.0 µg/mL Fluorescent DNA Dye (mL)	100 µg/mL DNA Stock (µL)	1.0 mg/mL DNA Stock (µL)	Final DNA Concentration (ng/mL)
1	2	0	-	0
2	2	2	-	100
3	2	5	-	250
4	2	10	-	500
5	2	-	2	1,000
6	2	-	5	2,500
7	2	-	10	5,000

8. Repeat Step 7 until all of the samples have been monitored.
9. Prepare a standard curve using the seven known DNA samples by plotting DNA concentration versus relative fluorescence units (RFU). See image below.
10. Determine the equation of the line using least squares regression.

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.om

AMRESCO, LLC

Corporate Headquarters
6681 Cochran Road
Solon, Ohio USA 44139-4300

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

Fluorescent DNA Quantitation Kit

ZY0548
Rev. 1 2/2013