Rev. 0

dsDNA Quantification Assay-Fluorometric (DQA-F)

Catalog #8828
500 reactions

Product Description

The ScienCell dsDNA Quantification Assay (DQA-F) is an ultra-sensitive fluorescence-based assay designed to accurately measure dsDNA concentration in solution. This simple and reliable protocol enables precise quantification of dsDNA in unknown samples, making it ideal for applications such as library preparation and cDNA synthesis. The assay can detect as little as 100 pg/mL of dsDNA and is optimized for high-throughput analysis using a 96-well plate format with fluorescence microplate reader. The included dye is highly selective for dsDNA, ensuring minimal interference from RNA or ssDNA. The assay provides a linear detection range from 250 pg/mL to 1000 ng/mL with the same dye dilution.

Kit Components

Cat #	Component	Quantity	Storage
8828a	20X TE Buffer	10 mL	4°C
8828b	dsDNA Dye 200X	250 µL	-20°C
8828c	Standard DNA	1 vial	-20°C

Additional Materials Required (Materials Not Included in Kit)

96-well plate
Nuclease free water
Fluorescent Plate-reader (excitation at ~480 nm and emission ~530 nm wavelengths)

Quality Control

Standards with concentrations ranging from 0.25 ng/mL to 1000 ng/mL is used to generate a standard curve R^2 value >0.98.

Product Use

FDQA is designed for rapid and sensitive detection quantitating dsDNA in solution
for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, store the dsDNA dye (Cat #8828b) in the dark at -20°C in a manual defrost freezer, and the 20x TE (Cat #8828a) at 4°C, and the Standard DNA (Cat #8828c) at -20°C in a manual defrost freezer. Aliquot dsDNA dye and freeze it and once thawed avoid freeze-thaw cycles and keep it at 4°C or on ice in the dark at all times.

Procedure

Note: *Prepare the reagent on the day of the experiment and allow all reagents to reach room temperature before use. Protect the working solution from light, as the dsDNA dye is susceptible to photodegradation. For optimal results, use the working solution within a few hours of preparation.*

A. **Reagent preparation**

1. Prepare 1X TE Buffer
   1.1. Dilute the 20X TE buffer with DI water.
   1.2. Example: Mix 500 µL of 20X TE buffer with 9.5 mL of DI water to prepare 1X TE buffer.
2. Prepare dsDNA Dye Working Solution
   2.1. Dilute the dsDNA Dye solution 200-fold in 1X TE buffer.
   2.2. For a 96-well plate with a total assay volume of 100 µL per well, each sample requires 90 µL of dsDNA Dye working solution.
   2.3. Example: To prepare enough working solution for an entire 96-well plate, mix 50 µL of dsDNA Dye with 9.95 mL of 1X TE buffer.

B. **Preparation of the DNA Standard Curve**

1. Dilute the 100 µg/mL standard DNA stock (Cat# 8828c) with 1X TE buffer to create either a high-range or low-range standard curve, depending on the concentration of your samples.
2. Use the dilution tables below to prepare the appropriate standard curve.
3. Add 10 µL of each prepared DNA standard into the designated wells.
4. Add 90 µL of dsDNA Dye working solution into each well

High-range standard curve		
TE Buffer	**DNA STD**	**Final DNA Concentration in Assay**
36 µL	4 µL (100 µg/mL)	1 µg/mL
36 µL	4 µL (10 µg/mL)	100 ng/mL
36 µL	4 µL (1 µg/mL)	10 ng/mL
40	0	Blank

Low-range standard curve		
TE Buffer	**DNA STD**	**Final DNA Concentration in Assay**
36 µL	4 µL (2.5 µg /mL)	25 ng/mL
36 µL	4 µL (250 ng/mL)	2.5 ng/mL
36 µL	4 µL (25 ng/mL)	0.25 ng/mL
40	0	Blank

C. **Preparation of DNA Samples**

1. Measure the samples DNA concentration by nanodrop and dilute the samples with TE buffer to a concentration within the expected range of the standard curve.
2. Load 10 µL the standard DNA samples and DNA samples into the wells of a 96 well plate, ensuring at least duplicates for each concentration.
3. Add 90 µL of dsDNA dye working solution to each well.
4. Include wells for blank and instead of dsDNA add 10 µL 1x TE buffer.
5. Incubate for 5 minutes in dark at room temperature.
6. Read the plate on plate-reader and set the excitation at ~480 nm and emission ~530 nm wavelengths.

D. **Data Analysis:**

1. Make a standard curve using the fluorescence values of the standard DNA concentrations. Check that your standard has R^2 value greater than 0.98.
2. Calculate the concentration of the DNA samples from the standard curve.
3. Assess the linearity and correlation coefficient of the standard curve.

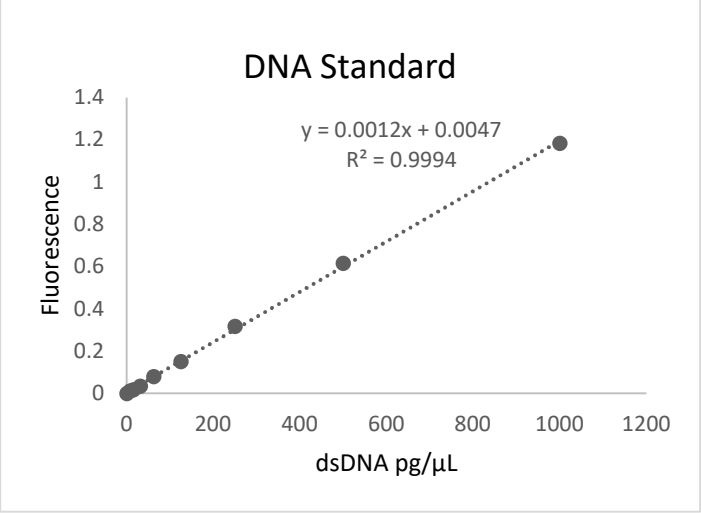

Figure 1. A typical of a DNA standard measured with dsDNA Quantification Assay.