dsDNA Quantification High Sensitivity Kit

(For use with microplate readers)

Catalog No. 40540H: 1000 assays
Storage: at 4°C and room temperature upon receive

Description

The **dsDNA Quantification High Sensitivity Kit** (For use with microplate reader) is developed for double stranded DNA quantification. The DNA Quantification HS kit includes HS Dye, HS Dilution Buffer, and DNA Standards. Simply dilute the HS Dye with the HS Dilution Buffer, add DNA sample, then read the concentration using microplate reader. The assay is accurate in the linear range from 0.2 to 100 ng, and is highly selective for double-stranded DNA over RNA (Figure 1).

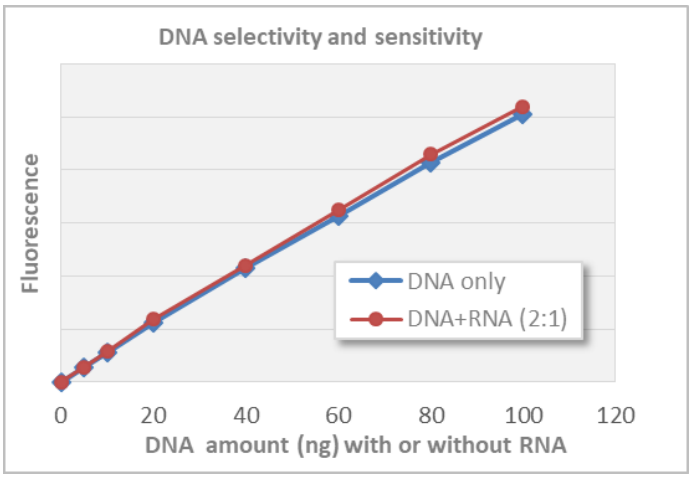

Fig. 1

Common contaminants such as salts, free nucleotides, solvents, detergents, RNA, single stranded DNA, or protein are well tolerated in the assay (Table 1).

Table 1. Effect of contaminants in the dsDNA HS Assay

Contaminant	Final concentration in the assay	Concentration in 10-µL sample	Result
Sodium chloride	10 mM	200 mM	OK
Magnesium chloride	5 mM	100 mM	OK
Sodium acetate	10 mM	200 mM	OK
Ethanol	1%	20%	OK
Phenol	0.10%	2%	OK
SDS	0.01%	0.20%	OK
Triton X-100	0.0025%	0.05%	OK
dNTP	100 µM	2 mM	OK
BSA	1 mg/mL	20 mg/mL	OK
RNA	0.5X	0.5X	OK
ssDNA	0.5X	0.5X	OK

Component

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- dsDNA HS Dilution Buffer 250 mL
- dsDNA HS Dye 1 mL
- dsDNA HS Standards (1 to 8) 0.5 mL each (0, 0.5, 1, 2, 4, 6, 8, 10 ng/µL)

Storage Condition

- dsDNA HS Dye (protect from light): store at 4°C, stable for 6 months.
- dsDNA HS Standards: store at 4°C, stable for 6 months.
- dsDNA HS Dilution Buffer: store at room temperature, stable for 6 months.

Reagent & Equipment Needed (not provided in this kit)

- Plastic container (disposable) for diluting the HS Dye with the HS Dilution Buffer
- Microplates for fluorescence-based assays

Assay Parameters

- **Assay temperature**: The assays are designed to be performed at room temperature (22-28°C). Store the Dilution Buffer at room temperature.
- **Incubation time**: To get optimal fluorescence, incubate the assay plate for 2 minutes after mixing the sample or standard with the working solution. The fluorescence signal is stable for 1 hour at room temperature after the incubation period.
- **Reagent photobleaching**: The reagent has high photostability with <0.3% fluorescence drop after 5 readings. However, if the assay tube remains in the fluorometer for multiple readings, the fluorescence will decrease as the temperature of solution will increase. If multiple readings of a single tube are needed, remove the tube from the fluorometer and wait for 30 seconds before taking next reading.

Protocol

1) Prepare the working solution by diluting the HS Dye 1:200 in HS Dilution Buffer in a clean plastic container (Do not use glass container). The volume for each well is 200 µL.
2) Add 200 µL of working solution into each well of the microplate.
3) Add 10 µL of each Standards (Standard 1 to Standard 8) into separate well of the microplate and mix by pipetting. Duplicates or triplicates of the standards are preferred.
4) Add 1-20 µL of samples into separate well of the microplate and mix by pipetting. Duplicates or triplicates of the samples are preferred.
5) Vortex the microplate using the plate reader if possible. Incubate the microplate at room temperature for 2 minutes after vortexing.
6) Measure the fluorescent value using microplate reader (Maximal excitation/emission are 495/540 nm; Standard fluorescent excitation/emission at ~480/530 nm are appropriate).
7) DNA amounts can be determined by the standard curve. Plot standards amount against fluorescence and fit a straight line to the points.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to BioDynami.com for Material Safety Data Sheet of the product.

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