

DNA Assay Kit (Fluorometric)

LS-K281-250 (250 Tests) • See Storage Conditions Below



Introduction

DNA quantitation is a common practice in molecular biology. Very often DNA is available in minute quantities and the traditional UV 260 nm absorbance Method requires microgram quantities for reliable results. Accurate determination of DNA concentration, especially when DNA is present at low concentrations, is crucial for reproducible results in sequencing, cloning, transfection and DNA labeling. Simple, direct and automation-ready procedures for measuring DNA concentration are very desirable. This DNA assay kit is designed to accurately measure nanogram quantities of plasmid DNA, cDNA, DNA following polymerase chain reaction and DNA eluted from gels. The improved Method utilizes Hoechst dye that binds specifically with double-stranded DNA. The fluorescence intensity, measured at $\lambda_{ex/em} = 350/450\text{nm}$, is directly proportional to the DNA concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

Key Features

- Sensitive and accurate. Linear detection range 2 ng to 40 ng (100 - 2,000 ng/mL) calf thymus DNA in 96-well plate assay.
- Simple and high-throughput. The "mix-and-read" procedure involves addition of a single working reagent and reading the fluorescence intensity. Can be readily automated as a high-throughput assay for thousands of samples per day.
- Low interference. RNA, salt (up to 3M NaCl), detergent (< 0.01% SDS) and common DNA extraction buffer do not interfere in the assay.

Applications

- Direct Assays: plasmid DNA, genomic DNA, cDNA, DNA following polymerase chain reaction, and DNA extracted from gel and other matrices.

Components

Component	K281-250
	250 Tests
Reagent	50 mL
Standard (10 $\mu\text{g/mL}$ calf thymus DNA)	1 mL

Materials Not Supplied

Pipetting devices and accessories. 1 x TE buffer.

Procedure using 96-well plate: Black 96-well plates (e.g. Corning) and fluorescence plate reader.

Procedure using cuvette: Fluorescence spectrophotometer and fluorometric cuvettes.

Storage

The kit is shipped at room temperature. Store the DNA standard at -20°C and the Reagent at $2-8^{\circ}\text{C}$. Shelf life: 12 months after receipt.

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Assay Procedure

Preparation: bring reagents to room temperature before use.

Procedure Using 96-Well Plate

1. Prepare 400 μ L 2000 ng/mL Premix by mixing 80 μ L Standard and 320 μ L TE buffer (10mM Tris, 1mM EDTA, pH 7.4). Dilute standards as follows. Transfer 20 μ L diluted standards and samples into wells of a black flat-bottom 96-well plate. Store standards at 4°C for future use.

No	Premix + TE	Vol (μ L)	DNA (ng/mL)
1	100 μ L + 0 μ L	100	2000
2	80 μ L + 20 μ L	100	1600
3	60 μ L + 40 μ L	100	1200
4	40 μ L + 60 μ L	100	800
5	30 μ L + 70 μ L	100	600
6	20 μ L + 80 μ L	100	400
7	10 μ L + 90 μ L	100	200
8	0 μ L + 100 μ L	100	0

2. Add 200 μ L working reagent and tap lightly to mix.
3. Incubate 1 min at room temperature and read fluorescence emission at 440 - 460nm (peak 450nm, excitation at 340-370 nm).

Procedure Using Cuvette

1. Transfer 100 μ L diluted standards and samples to cuvettes.
2. Add 1000 μ L working reagent and tap lightly to mix.
3. Incubate 1 min at room temperature and read fluorescence intensity at 440 - 460nm (peak 450nm, excitation at 340-360 nm).

Calculations

Subtract blank fluorescence value (water, #8) from the standard values and plot ΔF against standard DNA concentrations. Determine the slope using linear regression fitting. The DNA concentration of Sample is calculated as

$$= \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{\text{Slope}} \times n \quad (\text{ng/mL})$$

F_{SAMPLE} and F_{BLANK} are fluorescence values of the sample and water or buffer in which the sample was diluted, respectively. n is the sample dilution factor.

General Considerations

(1) For samples with unknown DNA concentration, pipet 1 μ L sample and mix with 99 μ L TE buffer. Make further serial 10-fold dilutions. Assay all diluted samples and choose dilutions at which the fluorescence intensity values fall within the linear calibration range to calculate sample DNA concentration. (2). Calf thymus DNA serves as a standard for plant and animal DNA, because the AT content is conserved among most DNAs from these two species. For bacterial DNA, a different standard should be used that best matches the sample DNA content. (3). Fluorescence intensity is half when binding to the same single-stranded DNA. Short single-stranded DNA pieces do not fluoresce with this dye.

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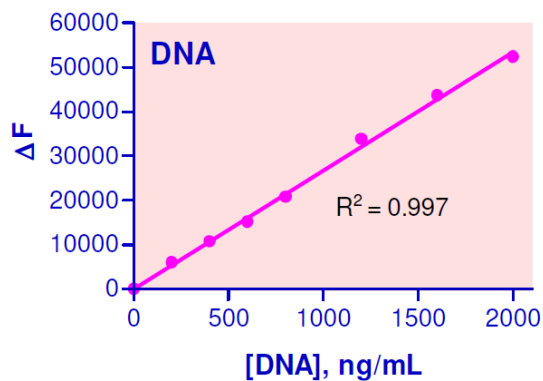
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Sample Data



Standard Curve in 96-well plate assay

Version: V.08.09.2018

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