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User's Manual and Instructions

DNA Assay Kit (Z5030023)

Fluorimetric Quantitation of Nanogram DNA

DESCRIPTION

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. Biochain's 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 450nm (λ_{exc} = 350nm), 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.

KIT CONTENTS (250 tests in 96-well plates)

Reagent: 50 mL Standard: 1 mL 10 μg/mL calf thymus DNA

Storage conditions. The kit is shipped at room temperature. Store the DNA standard at -20°C and the Reagent at 2-8°C. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Preparation: bring reagents to room temperature before use.

Procedure using 96-well plate:

1. Prepare 400 μL 2000 ng/mL Premix by mixing 80 μL Standard and 320 μL TE buffer (10mM Tris, 1mM EDTA, pH 7.4). Dilute standards as follows. Transfer 20 μL 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 (μL)	DNA (ng/mL)
1	100μL + 0μL	100	2000
2	80μL + 20μL	100	1600
3	60μL + 40μL	100	1200
4	40μL + 60μL	100	800
5	30μL + 70μL	100	600
6	20μL + 80μL	100	400
7	10μL + 90μL	100	200
8	0μL + 100μL	100	0

2. Add 200 μL 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 μL diluted standards and samples to cuvets.
2. Add 1000 μL 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).

CALCULATION

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

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

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories. 1 x TE buffer.

Procedure using 96-well plate:

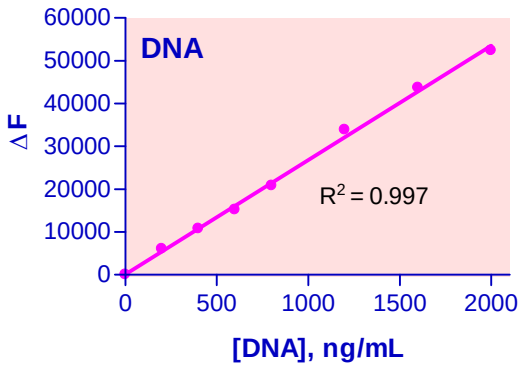
Black 96-well plates (e.g. Corning) and fluorescence plate reader.

Procedure using cuvette:

Fluorescence spectrophotometer and fluorometric cuvets.

GENERAL CONSIDERATIONS

(1) For samples with unknown DNA concentration, pipet 1 μL sample and mix with 99 μL 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.



Standard Curve in 96-well plate assay

LITERATURE

1. Bachoon DS, Otero E, Hodson RE (2001). Effects of humic substances on fluorometric DNA quantification and DNA hybridization. J Microbiol Methods 47:73-82.

2. Teare JM et al. (1997). Measurement of nucleic acid concentrations using the DyNA Quant and the GeneQuant. Biotechniques 22:1170-4.

3. Bester MJ, Potgieter HC, Vermaak WJ (1994). Cholate and pH reduce interference by sodium dodecyl sulfate in the determination of DNA with Hoechst. Anal Biochem. 223:299-305.