Technical Bulletin

DNA Assay Kit

Catalogue number MAK539

Product Description

DNA quantitation is a common practice in molecular biology. 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.

The 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.

The Linear detection range of the kit is 2 ng to 40 ng (100 – 2,000 ng/mL) calf thymus DNA in 96-well plate assay.

Components

The kit is sufficient for 250 fluorometric assays in 96-well plates.

- Reagent 50 mL

  Catalogue Number MAK539A

- Standard

  (10 µg/mL calf thymus DNA) 1 mL

  Catalogue Number MAK539B

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., multichannel pipettor)
- Fluorescence multiwell plate reader
- Black flat-bottom 96-well. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- TE buffer (10mM Tris, 1mM EDTA, pH 7.4)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store the DNA standard at -20 °C and the reagent at 2-8 °C once received.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate all components to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Note:

This procedure is written for 96-well plates.

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.

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.

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 Preparation

1. Prepare 2000 ng/mL Standard Premix: Mix 80 µL Standard and 320 µL TE buffer (10mM Tris, 1mM EDTA, pH 7.4) to make 400 µL of Standard Premix.
2. Dilute Standards as per Table 1.

Table 1.
Preparation of Standards

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

3. Transfer 20 µL diluted Standards and Samples into wells of a black flat-bottom 96-well plate.

Note: Standards can be stored at 4 °C for future use.

Measurement

1. Add 200 µL of Reagent and tap lightly to mix.
2. Incubate for 1 minute at room temperature.
3. Read fluorescence emission at 440 – 460 nm (peak 450 nm, excitation at 340-370 nm).

Results

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

DNA (ng/mL) =

$$\frac{F_{Sample}-F_{blank}}{Slope} \times n$$

Where:

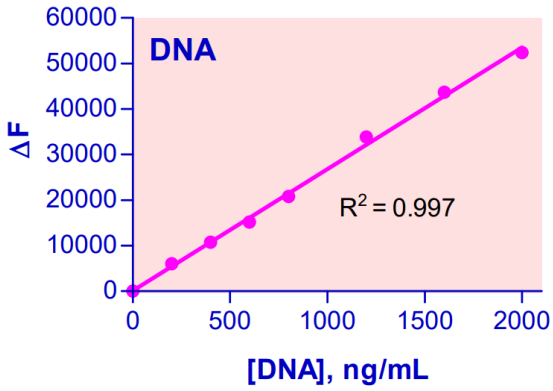
F_{SAMPLE} = Fluorescence values of the Sample.

F_{BLANK} = Fluorescence values of the Blank

Slope = Slope of the DNA Standard curve

n = Sample dilution factor.

Figure 1.
Typical DNA standard curve



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