dsDNA Quantification Kit – High Sensitivity (for microplate reader)

#GQ02.1000 (1000 assays)
(FOR RESEARCH ONLY)

Product: GRiSP's dsDNA Quantification Kits provide an easy and fast method for the quantification of double-stranded DNA (dsDNA). This high sensitivity (HS) kit was developed for use with microplate readers and is accurate and reliable over a linear range from 0.2ng to 100ng.

GRiSP's dsDNA Quantification Kits are highly specific for double-stranded DNA. Presence of single-stranded DNA (ssDNA) or RNA does not interfere with the measurements. Other commonly present contaminants in DNA samples, such as proteins, ethanol, phenol, free nucleotides, detergents (SDS, Triton X-100), and salts (NaCl, $MgCl_2$, acetate) also have little to no effect on the fluorescence signal.

Applications: Quantification of dsDNA over a (0.2-100ng) using a microplate reader

Contents: The dsDNA Quantification Kit – High Sensitivity (for microplate reader) contains sufficient reagents for 1000 assays.

Component	GQ02.1000
High Sensitivity Dye	1 ml
Dilution Buffer (HS)	250 ml
dsDNA standards (8 in total) *	0.5 ml (each)

* (concentrations of 0, 0.5, 1, 2, 4, 6, 8 and 10ng/µl)

Properties:
Fast, Accurate and Reliable
Broad Range from 0.2ng to 100ng
Highly Specific for dsDNA

Storage: Store the High Sensitivity Dye and the dsDNA standards at +4ºC, protected from light, for up to 1 year. Dilution Buffer can be stored at either at room temperature or +4ºC for up to 1 year. Before usage, allow the Dilution Buffer to warm up to room temperature as the assay should be carried out at Rt (22-28ºC).

Protocol

1. Using a clean plastic tube/container, mix for each assay 1 µl of High Sensitivity Dye with 200 µl of Dilution Buffer (warmed up to room temperature). **Do not** use a glass container! (e.g. for 96 samples, mix 96 µl Dye with 19.2 ml Buffer).
2. Pipet 200 µl of the mixture into each well of a microplate suitable for fluorescence-bases assays (not included)
3. Add 10 µl of each of the 8 dsDNA standards, in duplicate or preferably in triplicate, in separate wells of the microplate and mix by pipetting.
4. Add 10 µl** of each of DNA sample, in duplicate or triplicate, in separate wells of the microplate and mix by pipetting.
   **Instead of 10 µl, one could add any volume between 1 µl and 20 µl of DNA sample. However, one should adjust for this when determining the initial concentration of the dsDNA in the sample when using the obtained standard curve.
5. If possible, vortex the microplate using the microplate reader. Before starting measurements, incubate the plate at room temperature for 2 minutes, to get optimal fluorescence. The fluorescence signal is stable for 1 hour after the incubation.
6. Measure fluorescence using a microplate reader.
   Max Excitation/Emission: 485/545nm whereas Standard Fluorescent Excitation/Emission at ~480/530nm are suitable.
7. Prepare a calibration curve (standard curve) by plotting fluorescence signals against the corresponding dsDNA standards and drawing a line that best fits the data points (should be a straight line).
8. dsDNA concentrations of test samples can be determined by direct comparison of the fluorescence signal obtained in the corresponding well with the standard curve. In case of having added another amount than 10µl of sample in step 4, this should be taken into account for the determination of the initial concentration.