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QuDye dsDNA HS Assay Kit manual

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QuDye dsDNA HS Assay Kit manual

The kit is used for quantification of dsDNA with Fluorometer.
QuDye dsDNA HS reagent selectively binds to double-stranded DNA, so nucleotides, single-stranded DNA, RNA, proteins, and other impurities do not impede the measurements. All reagents are optimized to perform the measurements with fluorometer, the measurement range of initial sample DNA concentrations is from 10 pg/µL to 100 ng/µL.

Kit components

Kit component	Count			
	12102 100 assays	13102 100 assays	52102 500 assays	53102 500 assays
33010, QuDye dsDNA HS Reagent, 200x, 250 uL	1	1	—	—
B9650, Quantitative standard, 0 ng/uL in TE buffer, 1 mL	1	1	—	—
B7650, dsDNA quantitative standard, 10 ng/uL in TE buffer, 1 mL	1	1	—	—
G2150, TE buffer, 20x, 5 mL	1	1	—	—
33115, Polypropylene tube (0.5 mL thin-walled transparent), 100 pcs	—	1	—	5
63010, QuDye dsDNA HS Reagent, 200x, 1.25 mL	—	—	1	1
G9650, Quantitative standard, 0 ng/uL in TE buffer, 5 mL	—	—	1	1
G7650, dsDNA quantitative standard, 10 ng/uL in TE buffer, 5 mL	—	—	1	1
N2150, TE buffer, 20x, 25 mL	—	—	1	1

Store at 4 °C. Warm to RT before use.

Shelf life 12 months.

! All measurements with QuDye dsDNA HS Assay Kit should be performed at room temperature (22–28 °C). Before starting, equilibrate all kit's solutions to room temperature. Avoid warming the samples, as the sample temperature influences the measurement results; particularly do not hold the assay tubes in your hands just before fluorescence measurement with a fluorometer.

Protocol

1. Prepare 1x TE buffer taking into account that 200 µL of 1x TE buffer will be required for each sample and for each of the two standards. In order to do that, dilute 20x *TE concentrate* 20-fold with deionized water.
2. Prepare *QuDye dsDNA HS dye working solution* taking into account that 200 µL of dye working solution will be required for each sample and for each of the two standards. In order to do that, dilute *QuDye dsDNA HS reagent* concentrate 200-fold with *1x TE buffer*.

   For example, to measure 3 samples and 2 standards, prepare 200 µL x 5 = 1000 µL of 1x TE buffer and 1000 µL of dye working solution (mix 5 µL of QuDye dsDNA HS reagent concentrate and 995 µL of 1x TE buffer).

   ! It is recommended to use dye working solution within several hours after preparation. In case of postponed measurements protect prepared dye working solution from light.

   ! Use only plastic containers to prepare dye working solution, as QuDye dsDNA HS reagent can adsorb to glass surfaces, which results in decreasing of the dye concentration in samples and biases in the measurement results.
3. Set up two 0.5 ml tubes (thin-walled and optical-transparent) for the standards and one tube for each sample. Label the tube lids. Do not label the side of the tube as this can interfere with the sample read.
4. To each of two tubes for standards add 190 µL of *QuDye dsDNA HS dye working*

solution and either 10 µL of *Quantitative standard, 0 ng/µL (Standard #1)* or *dsDNA quantitative standard, 10 ng/µL (Standard #2)*. Vortex for 2–3 seconds and centrifuge briefly.

5. To each tube for samples add 180–199 µL of *QuDye dsDNA HS dye working solution* and 20–1 µL of DNA sample, respectively (the total volume should be 200 µL). Vortex for 2–3 seconds and centrifuge briefly.

   Dilution of the experimental sample is optional and depends on its initial concentration. The initial sample concentration can vary from 10 pg/µL to 100 ng/µL; however, after diluting with QuDye dsDNA HS dye working solution the amount of DNA should correspond to the measurement range of fluorometer (0.2–100 ng of DNA in 200 µL of the test sample). Therefore, a sample with the minimal acceptable initial DNA concentration (10 pg/µL) should be diluted 10-fold to 1 pg/µL [put 180 µL of dye working solution and 20 µL of the sample (10 pg/µL) in the assay tube, which corresponds to 0.2 ng of DNA]. A sample with the maximal acceptable initial DNA concentration (100 ng/µL) should be diluted 200-fold [put 199 µL of dye working solution and 1 µL of the sample (100 ng/mL) in the assay tube, which corresponds to 100 ng of DNA]. However, avoid using too small volumes when diluting the initial sample in order to maintain accuracy and precision of your measurements.

6. Incubate all tubes (containing standards and DNA samples) for 3–5 minutes at room temperature.

7. Perform the fluorescence measurements.

Fluorescence measurement with a fluorometer

The next steps should be carried out according to the manual of the fluorometer. Depending on the version of the fluorometer the menu items may differ from the specified below.

1. On the Home screen of the fluorometer, choose **DNA** as the assay type, then **dsDNA High Sensitivity**.
2. The software will automatically switch to the **Standards** tab. It is recommended to run fluorometer calibration whenever preparing a new dye working solution. You can use the previous calibration that you have performed before if all experiment conditions, including temperature in your laboratory, remain unchanged. In this case, press **No** on the **Standards** tab, and the software will switch to the **Sample** tab to measure fluorescence of the experimental samples. Proceed to item 3.

   To run new calibration, press **Yes** in the **Standards** tab. Insert the tube containing Standard #1 into the sample chamber, close the lid, then press **Read**. When reading is complete (~3 seconds), remove Standard #1. Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read**. When reading is complete, remove Standard #2. When calibration is complete, the software will proceed to the **Sample** tab to measure fluorescence intensity of the experimental samples.
3. In the **Sample** tab, insert the tube containing the experimental sample into the sample chamber, close the lid, then press **Read**. When measurement is complete, the software will show the QF value on the screen.

   The QF value is DNA concentration after dilution of the initial sample in the assay tube. Calculate the initial DNA concentration using the formula:

   Concentration of DNA in the initial sample (ng/mL) = QF value × 200/V, where

   - V (µL) is volume of the initial sample that was added to the assay tube (1–20 µL);
   - QF is the measurement result on the fluorometer screen (ng/mL).

Repeat the procedure for all experimental samples.

To calculate DNA concentration in the initial sample you can also use **Dilution Calculator** in the fluorometer.

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