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Life science solutions

QuDye ssDNA Assay Kit manual

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QuDye ssDNA Assay Kit manual

The kit is intended for quantification of single-stranded DNA (ssDNA) with fluorometer. With this kit you can measure concentration of either oligonucleotide or long ssDNA; free nucleotides do not bind to the dye and do not affect measurement results. QuDye ssDNA reagent does not have selectivity to single-stranded DNA and can also bind to double-stranded DNA (dsDNA) and RNA, so they should be avoided in the sample. Other minor contaminants such as salts, detergents, solvents, and proteins have non-significant effect on measurements results (Table 1), however, it is recommended to minimize or completely eliminate them in the sample.

All reagents are optimized to perform the measurements with fluorometer. The range of ssDNA concentrations measured is from 50 pg/µL to 200 ng/µL for initial sample (final DNA content in the assay tube after diluting the sample in the dye working solution is 1–200 ng).

Kit components

Kit component	Count	
	17102 100 assays	18102 100 assays
33115, Polypropylene tube (0.5 mL thin-walled transparent), 100 pcs	—	1
35010, QuDye ssDNA Reagent, 200x, 250 uL	1	1
S3250, TE buffer, 1x, 50 mL	1	1
B9650, Quantitative standard, 0 ng/uL in TE buffer, 1 mL	1	1
B2650, ssDNA quantitative standard, 20 ng/uL in TE buffer, 1 mL	1	1

Store at +4 °C. Warm to RT before use.

Shelf life 12 months.

! All measurements with QuDye ssDNA Assay Kit should be performed at room temperature (22–28 °C). Before starting, equilibrate all kit's solutions to room temperature. When using the kit on a regular basis, store QuDye ssDNA Reagent and 1x TE buffer at room temperature, standards — at +4 °C.

! Please note that fluctuations in sample temperature can significantly affect measurement results. Avoid warming the samples; particularly do not hold the assay tubes in your hands just before fluorescence measurement with a fluorometer. If being in the fluorometer chamber even for a short time, the tube with the sample gets warmer, so perform measurements just after placing the tube with the sample in the fluorometer chamber. If one sample has to be reread, the tube with the sample should be removed from the fluorometer just after reading and placed in the fluorometer chamber only when fluorescence is measured.

Protocol

1. Prepare *QuDye ssDNA 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 ssDNA 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 dye working solution (mix 5 µL of QuDye ssDNA 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 ssDNA reagent can adsorb to glass surfaces, which results in decreasing of the dye concentration in samples and biases in the measurement results.

2. 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.

3. To each of two tubes for standards add 190 µL of *QuDye ssDNA dye working solution* and either 10 µL of *Quantitative standard, 0 ng/µL (Standard #1)* or *ssDNA quantitative standard, 20 ng/µL (Standard #2).* Vortex for 2–3 seconds and centrifuge briefly.

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

   The dilution of test sample is optional and depends on its initial concentration. The initial sample concentration should be within the range of 50 pg/µL—200 ng/µL. Final DNA content in the assay tube after diluting the sample in the dye working solution should be within the range of 1–200 ng for measurement with a fluorometer. At the same time, avoid pipetting small volumes to dilute the initial sample in order to maintain accuracy and precision of your measurements.

5. Incubate all tubes (containing standards and DNA samples) for 2 minutes at room temperature.
6. Perform the fluorescence measurements.

Fluorescence measurement with a fluorometer

The next steps should be carried out according to the instruction 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 **ssDNA** as the assay type. Press **Go**.
2. With each preparation of the dye working solution, calibrate the fluorometer. Select **Run new calibration** and press **Go**.
3. Insert the tube containing *Standard #1* into the sample chamber, close the lid, then press **Go**. When the reading is complete (~3 seconds), remove *Standard #1*. Insert the tube containing *Standard #2* into the sample chamber, close the lid, then press **Go**. When the reading is complete, remove *Standard #2*.
4. Insert the tube containing user sample into the sample chamber, close the lid, then press **Go**. On the screen you will see the QF value.

   Calculate the concentration of the sample by the formula: Concentration of the sample = QF value x 200/sample volume.

Table 1. Effect of contaminants on fluorescence when measuring ssDNA concentration with the QuDye ssDNA Assay Kit

Contaminants	Final concentration in test sample	Change in fluorescence (increase ↑ or decrease ↓)	
Sodium acetate	30 mM	↓	Non-significant (less than 10%)
Agarose	0.1%	↑	
Chloroform	2%	↑	
Ammonium acetate	50 mM	↓	Moderate (10-20%)
Phenol	0.2%	↓	
Ethanol	10%	↑	
Triton X-100	0.1%	↑	
Bovine serum albumin	2%	↑	Significant (more than 20%)
IgG	0.1%	↓	
Sodium chloride	100 mM	↓	
Zinc chloride	1 mM	↓	
Magnesium chloride	5 mM	↓	
Urea	2 M	↑	
Sodium dodecyl sulfate	0.01%	↑	
Polyethylene glycol	1%	↑	
ATP	0.1%	↑	

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