Lumiprobe
Life science solutions

QuDye® dsDNA BR Assay Kit manual

Lumiprobe Corporation. All rights reserved.

Contents

English: QuDye® dsDNA BR Assay Kit manual 3-7

QuDye® dsDNA BR Assay Kit manual

QuDye® dsDNA BR Assay Kit (Broad Range) is intended for quantification of double-stranded DNA using fluorometer. QuDye® dsDNA BR Reagent selectively binds to double-stranded DNA, so any RNA, single-stranded DNA, free nucleotides, or protein contaminants in the sample do not alter measurement results. Other minor contaminants such as salts, detergents, and solvents have a non-significant effect on measurements results. However, it is recommended to minimize or completely eliminate them in the sample. All the reagents are optimized for operations with fluorometer (all its versions) in the range of initial DNA concentrations from 100 pg/μL to 1,000 ng/μL (the final amount of DNA after dilution of the initial sample is 2–1,000 ng in 200 μL of the test sample). All measurements are performed at room temperature; fluorescence signal of samples is stable for 3 hours.

To determine the protein contaminants in the DNA sample, you can use QuDye® dsDNA BR Assay Kit with QuDye® Protein Quantification Kit.

Kit components

Kit component	Count						
	A9102 10 assays	19102 100 assays	1Z102 100 assays	59102 500 assays	69102 500 assays	89102 1000 assays	79102 1000 assays
28010, QuDye® dsDNA BR Reagent, 200×, 30 uL	1	—	—	—	—	—	—
AA650, dsDNA quantitative standard, 100 ng/uL in TE buffer, 100 uL	1	—	—	—	—	—	—
K9650, Quantitative standard, 0 ng/uL in TE buffer, 10 mL	—	—	—	—	—	1	—

KA650, dsDNA quantitative standard, 100 ng/uL in TE buffer, 10 mL	—	—	—	—	—	1	—
B9650, Quantitative standard, 0 ng/uL in TE buffer, 1 mL	1	1	1	5	5	—	10
S4850, QuDye BR Buffer, 1x, 50 mL	1	1	1	5	5	10	10
38010, QuDye® dsDNA BR Reagent, 200×, 250 uL	—	1	1	—	—	—	—
BA650, dsDNA quantitative standard, 100 ng/uL in TE buffer, 1 mL	—	1	1	5	5	—	10
33115, Polypropylene tube (0.5 mL thin-walled transparent), 100 pcs	—	—	1	—	5	—	—
68010, QuDye® dsDNA BR Reagent, 200×, 1.25 mL	—	—	—	1	1	2	2

Store at 4 °C. Warm up to room temperature before use. Transportation: at room temperature for up to 3 weeks.

Shelf life 12 months.

! All measurements with QuDye® dsDNA BR 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® dsDNA BR Reagent and QuDye® BR 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® dsDNA BR 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 *200× QuDye® dsDNA BR Reagent concentrate* 200-fold with *QuDye® BR Buffer*.

   For example, to measure 3 samples and 2 standards, prepare 200 µL × 5 = 1,000 µL of dye working solution (mix 5 µL of QuDye® dsDNA BR Reagent concentrate and 995 µL of QuDye® BR 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 BR 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® dsDNA BR dye working solution* and either 10 µL of *Quantitative standard, 0 ng/µL (Standard #1)* or *dsDNA quantitative standard, 100 ng/uL (Standard #2)*. Vortex for 2–3 seconds and centrifuge briefly.

4. To each tube for samples add 180–199 µL of *QuDye® dsDNA BR dye working solution* and 20–1 µL of test sample, respectively (the total volume in each tube should be 200 µL). Vortex for 2–3 seconds and centrifuge briefly. Make sure that bubbles have not been formed in the tube. If necessary, remove bubbles by centrifugation.

   Dilution of the experimental sample is optional and depends on its initial concentration. The initial sample concentration can vary from 100 pg/µL

   to 1,000 ng/µL; however, after diluting with QuDye® dsDNA BR dye working solution the amount of DNA should correspond to the measurement range of fluorometer (2–1,000 ng of DNA in 200 µL of the test sample). Therefore, a sample with the minimal acceptable initial DNA concentration (100 pg/µL) should be diluted 10-fold to 10 pg/µL [put 180 µL of dye working solution and 20 µL of the sample (100 pg/µL) in the assay tube, which corresponds to 2 ng of DNA]. A sample with the maximal acceptable initial DNA concentration (1,000 ng/µL) should be diluted 200-fold [put 199 µL of dye working solution and 1 µL of the sample (1,000 ng/mL) in the assay tube, which corresponds to 1,000 ng of DNA]. However, avoid using too small volumes when diluting the initial sample in order to maintain accuracy and precision of your measurements.

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

6. Perform the fluorescence measurements.

Fluorescence measurement with a fluorometer

The next steps should be carried out according to the manual of 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 Broad Range**.

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.

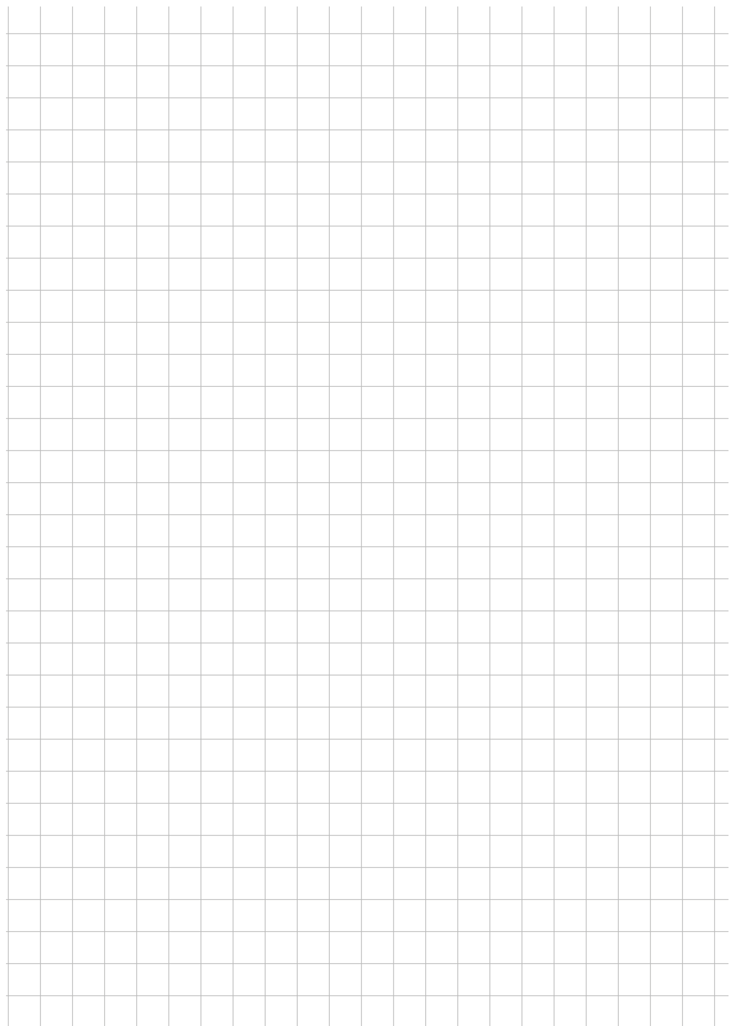
   The QF value is DNA concentration after dilution of the initial sample in the assay tube. Calculate the initial DNA concentration using the formula:

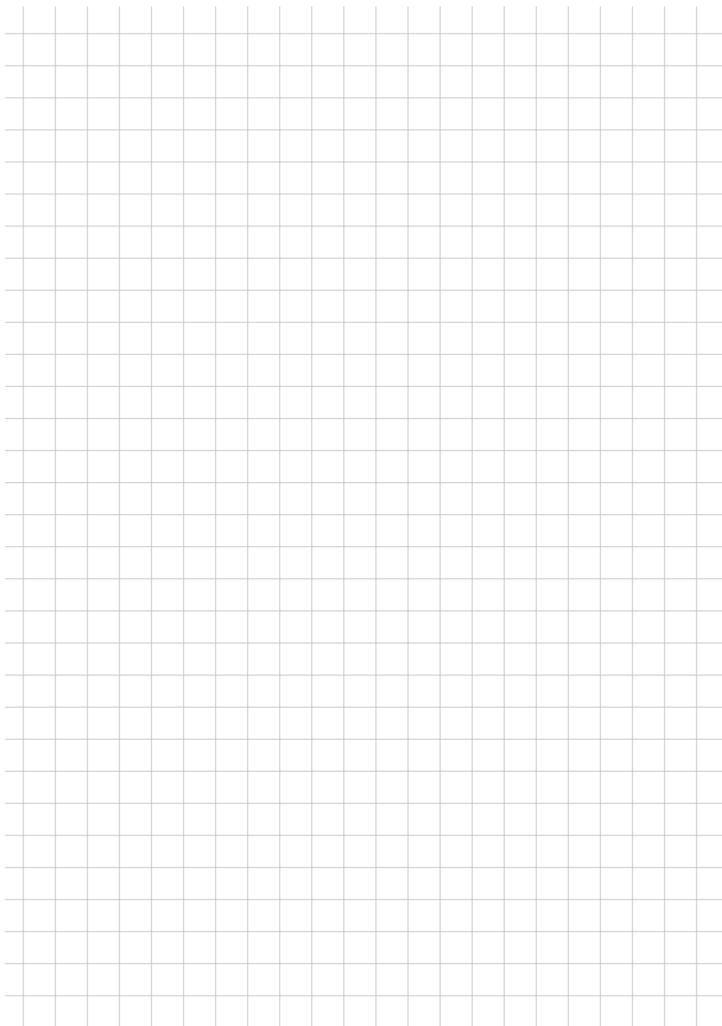
   Concentration of DNA in the initial sample (µg/mL) = QF value × 200/V, where

   - 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 (µg/mL).

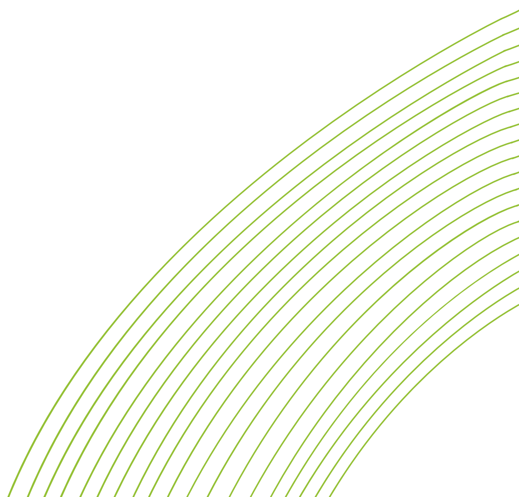
   Repeat the procedure for all experimental samples.

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





22.09.509-QM
Issued by INSPECT



www.lumiprobe.com