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Life science solutions

QuDye Protein Quantification Kit manual

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The kit is used for the quantification of proteins with Fluorometer. QuDye Protein Reagent selectively binds to SDS-protein micelles, so nucleotides, DNA, RNA and other impurities (but not detergents) do not impede the measurements. All reagents are optimized to perform the measurements range of initial protein concentrations is 12.5–5000 µg/mL (the final amount of protein after dilution of the initial sample is 0.25-5 µg in 200 µL of the test sample). The assay is highly sensitive and widely applicable due to low fluctuations of fluorescence intensity during quantification of various proteins. The kit includes all the necessary components. Measurements with the kit do not require any special sample preparation (including pre-heating at 95 °C). All measurements are performed at room temperature and take on average 30 minutes for 5–10 samples.

Kit components

Kit component	Count	
	15102 100 assays	25102 500 assays
15210, QuDye Protein Quantification Reagent, 200×, 100 uL	3	—
55210, QuDye Protein Quantification Reagent, 200×, 1.5 mL	—	1
S1750, QuDye Protein buffer, 1x, 50 mL	1	3
B3650, Protein standard, 0 ng/uL, TE buffer, 1 mL	1	1
B4650, Protein standard, BSA 200 ng/uL in TE buffer, 1 mL	1	1
B5650, Protein standard, BSA 400 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 Protein Quantification 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 *QuDye Protein dye working solution* taking into account that 200 µL of dye working solution will be required for each sample and for each of the three standards. In order to do that, dilute *QuDye Protein Reagent* concentrate 200-fold with *QuDye Protein buffer*.

   For example, to measure 2 samples and 3 standards, prepare 200 µL x 5 = 1000 µL of dye working solution (mix 5 µL of QuDye Protein Reagent concentrate and 995 µL of QuDye Protein 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 Protein 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 three 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. Add 190 µL of *QuDye Protein dye working solution* to each of three tubes used for standards and 10 µL of each *Protein standard* to the appropriate tube. Vortex for 2–3 seconds and centrifuge briefly.

4. Add 180–199 µL of *QuDye Protein dye working solution* to each of the tubes used for samples and 20–1 µL of protein 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 12.5 to 5000

µg/mL; however, after diluting with QuDye Protein dye working solution the amount of protein should correspond to the measurement range of fluorometer (0.25-5 µg of protein in 200 µL of the test sample). Therefore, a sample with the minimal acceptable initial protein concentration (12.5 µg/mL) should be diluted 10-fold to 1.25 µg/mL [put 180 µL of dye working solution and 20 µL of the sample (12.5 µg/mL) in the assay tube, which corresponds to 0.25 µg of protein]. A sample with the maximal acceptable initial protein concentration (5000 µg/mL) should be diluted 200-fold [put 199 µL of dye working solution and 1 µL of the sample (5000 µg/mL) in the assay tube, which corresponds to 5 µg of protein]. However, avoid using too small volumes when diluting the initial sample in order to maintain accuracy and precision of your measurements.

5. Incubate all tubes (containing standards and protein samples) for 15 minutes at room temperature.
6. Perform the fluorescence measurements.

Fluorescence measurement with a fluorometer

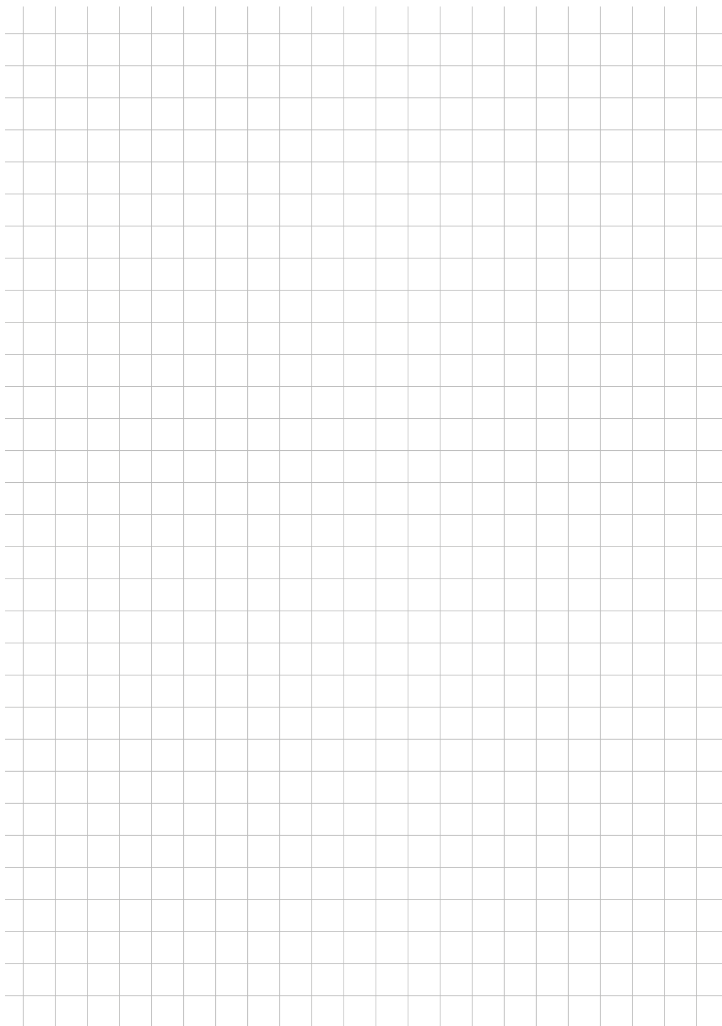
The next steps should be carried out according to the instruction of the fluorometer. Depending on the version of the fluorometer the menu items may differ from the specified below.

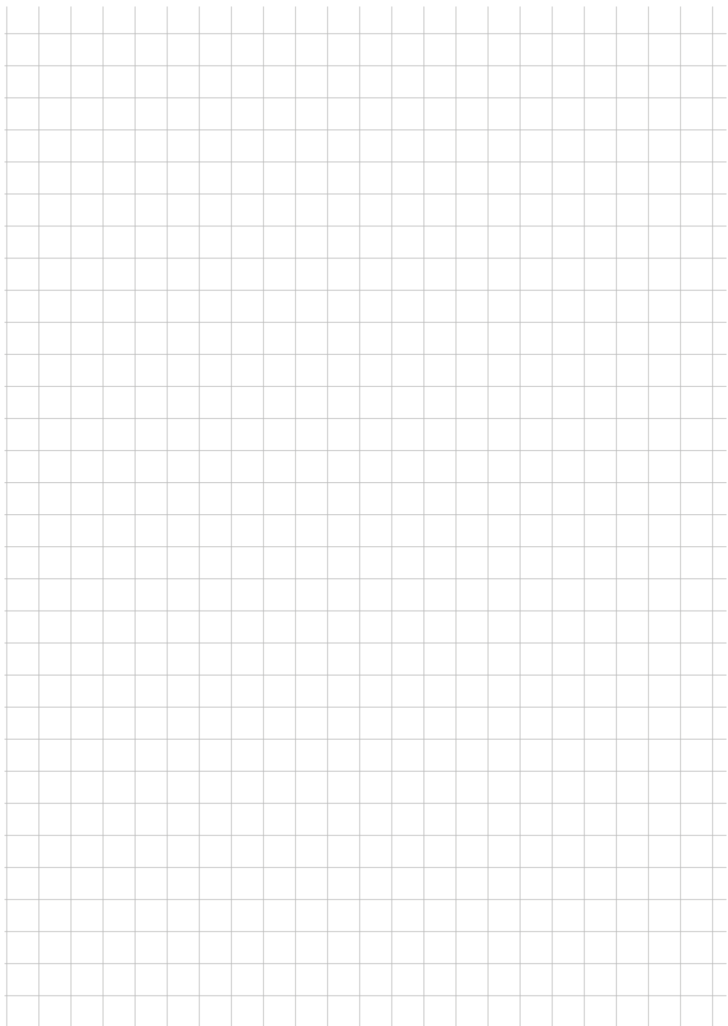
1. On the Home screen of the fluorometer, choose «Quant – It Protein» as the assay type. Press «Go»
2. With each preparation of the dye working solution, calibrate the fluorometer. Select «Run new calibration» and press «Go».
3. Insert the tube containing *Standard #1 (0 ng/uL, TE buffer)* into the sample chamber, close the lid, then press «Go». When the reading is complete (~3 seconds), remove *Standard #1*.

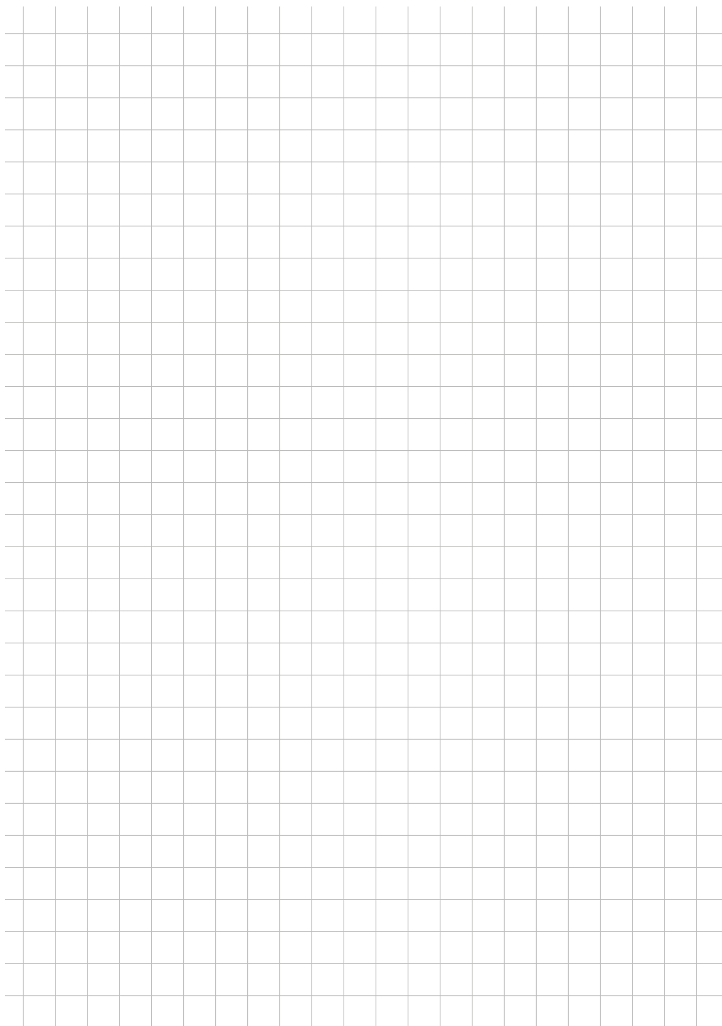
   Insert the tube containing *Standard #2 (BSA 200 ng/uL in TE buffer)* into the sample chamber, close the lid, then press «Go». When the reading is complete, remove *Standard #2*.

   Insert the tube containing *Standard #3 (BSA 400 ng/uL in TE buffer)* into the sample chamber, close the lid, then press «Go». When the reading is complete, remove *Standard #3*.
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 protein sample by the formula: Concentration of the sample = QF value x 200/sample volume.







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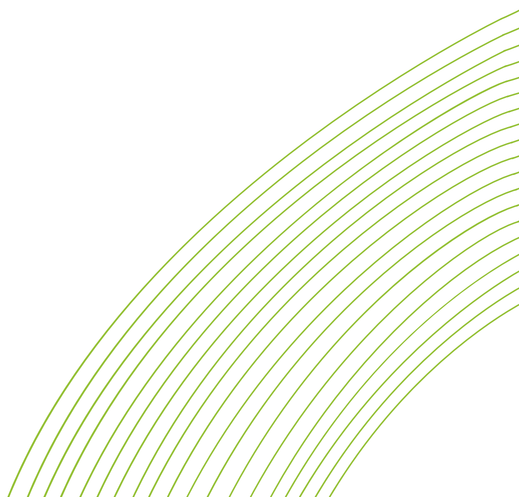

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