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ProteOrange® Protein Quantification Kit manual

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ProteOrange® Protein Quantification Kit manual

The kit is intended for highly sensitive protein quantification by fluorescence-based assay. Free ProteOrange® reagent has a very low ability to fluoresce. It selectively binds to protein molecules thus forming complexes in which it exhibits significant fluorescence when exposed to blue light. Fluorescence-based protein quantification with ProteOrange reagent is more sensitive than spectrophotometry or other conventional methods that are used for protein quantification (Lowry, Bradford, BCA).

The ProteOrange/protein complex exhibits fluorescence with an excitation maximum at ~485 nm and an emission maximum at ~590 nm. The kit can be used with any fluorometer that has a suitable excitation source and detection channel. The range of concentrations that can be measured is 10 ng/mL to 10 µg/mL for cuvette fluorometers and 100 ng/mL to 10 µg/mL for plate fluorometers.

Kit components

Kit component	Count
	14102 1 mL dye
41210, ProteOrange® Protein Quantification Reagent, 500×, 1 mL	1
A6650, Protein standard, BSA 2 mg/mL in TE buffer, 500 uL	1
S2750, ProteOrange protein quantification buffer, 10x, 50 mL	1

Store at 4 °C. Warm up to 20 °C before use.Transportation: at room temperature for up to 3 weeks.

Shelf life 12 months.

Protocol

This protocol is for the case when the kit is used with a plate fluorometer, and assay volume of 200 µL (96-well plate). If a fluorometer with another assay volume is used (for example, a cuvette fluorometer with an assay volume of 2 mL for a standard fluorometric cuvette), recalculate all volumes accordingly.

The protocol for this kit includes sample heating at 90 to 95 °C for 10 minutes for protein denaturation and stabilization of fluorescence signal in the test samples. The protocol below specifies that protein standards and test samples should be prepared in individual tubes, then heated and transferred onto the plate to measure fluorescence. If relevant equipment is available, you can prepare protein standards and test samples on the plate, tightly cover the plate with a lid or film resistant to high temperatures to prevent evaporation, and heat the samples according to the protocol. When the samples are cooled down to room temperature, discard the condensate by centrifugation and proceed according to the protocol.

1. **Preparation of 1× ProteOrange buffer.**

   Prepare the sufficient amount of 1× ProteOrange buffer according to the sample volume and amount of test samples (see item 4) and protein standards (see item 3). To prepare 1× buffer, dilute *10× ProteOrange buffer concentrate* 10-fold with deionized water.

2. **Preparation of ProteOrange dye working solution.**

   Prepare *ProteOrange dye working solution*. In order to do that, dilute *500× ProteOrange reagent concentrate* 500-fold with *1× ProteOrange buffer*.

   For example, to quantify 3 samples (see item 4, example #1) and 10 standards (see item 3), you should prepare about ~4 mL of 1× ProteOrange buffer and 4 mL of dye working solution (mix 8 µL of ProteOrange reagent concentrate with 4 mL of 1× ProteOrange buffer).

   ! The dye working solution should be used within a few hours after its preparation. In case of postponed measurements protect the prepared dye working solution from light.

! Prepare the dye working solution in plastic containers only. The dye can adsorb to glass surfaces, which results in decreasing in the dye concentration in the samples and biases in the measurement results.

3. **Preparation of protein standards.**

   Protein concentration standards are required to generate a calibration curve, which is used to convert fluorescence intensity of the test sample to its protein concentration. The calibration curve makes allowance for result variability when different fluorometers are used, between different experimental runs using the same fluorometer, and for pipetting errors during preparation of the dye working solution. Because of this, it is recommended to generate a calibration curve for every new run of experiments; however, you can use the calibration curve generated in previous experiments if experiment conditions remain the same.

   To generate a calibration curve, it is preferable to use the same protein that will be quantified in the test samples. If the same protein cannot be used, use protein concentration reference standard from the kit (i. e. BSA solution 2 mg/mL).

   The range of concentrations that can be measured with this kit is 10 ng/mL to 10 µg/mL for cuvette fluorometers and 100 ng/mL to 10 µg/mL for plate fluorometers. Depending on the expected protein concentration in the test samples and the fluorometer used, you can generate a calibration curve either for the whole working range of the kit or for its part.

   Please see below for the procedure for preparation of BSA concentration standards for the whole working range of the kit for plate fluorometer (100 ng/mL to 10 µg/mL) and assay volume of 200 µL (for 96-well plate):

   - 3.1. Using the *protein standard, BSA 2 mg/mL* from the kit, prepare BSA stock solutions 10 µg/mL and 1 µg/mL in ProteOrange dye working solution in individual 1.5 mL tubes.
     - 10 µg/mL: 5 µL of protein standard, BSA 2 mg/mL in TE buffer + 995 µL of ProteOrange dye working solution
     - 1 µg/mL: 100 µL of BSA stock solution 10 µg/mL + 900 µL of ProteOrange dye working solution
   - 3.2. Prepare BSA standards using prepared BSA stock solutions in individual 1.5 mL tubes according to the table below:

BSA stock solution*	Volume of BSA stock solution, µL	Volume of ProteOrange dye working solution, µL	Final BSA concentration, µg/mL
	0	250	0
10 µg/mL	250	0	10
	200	50	8
	100	150	4
	50	200	2
1 µg/mL	250	0	1
	200	50	0.8
	100	150	0.4
	50	200	0.2
	25	225	0.1

* Stock solutions prepared in item 3.1 in ProteOrange dye working solution

If expected concentrations of the test samples are in the known narrower range, you can prepare one BSA stock solution and respective BSA standards so that the calibration curve covers the expected range of test protein concentrations. For example, if expected concentration of the test sample is from 2 to 10 µg/mL, you should prepare only one BSA stock solution (10 µg/mL) (see item 3.1) and then BSA standards from it with concentrations of 0, 10, 8, 4 and 2 µg/mL (according to the table, see item 3.2) for measurements on the fluorometer.

4. **Preparation of test samples**

Dilute a protein sample with ProteOrange dye working solution in an individual tube. We recommend diluting the initial protein sample more than 20-fold so that the volume of the initial sample in the final sample measured does not exceed 5 %. This is caused by the dye amount that binds to the protein in the sample and the highest dilution of contaminants from the initial sample, which neutralizes their effect on results.

Example #1: To prepare one sample with a volume of 200 µL, mix 5 µL of initial protein sample and 195 µL of ProteOrange dye working solution (1:40 dilution).

Example #2 (for high concentration of contaminants in the initial protein sample): Prepare two sequential dilutions of the initial protein sample with ProteOrange dye working solution:

- 20× dilution: Mix 13 µL of initial protein sample with 247 µL of ProteOrange dye working solution.
- 100× dilution: Mix 50 µL of 20× diluted sample with 200 µL of ProteOrange dye working solution.

You will obtain two dilutions of the test protein (20× and 100×). If results are significantly biased due to high levels of contaminants in the initial protein sample, fluorescence intensities for the dilutions will not follow proportionally the calibration curve according to the dilution. In this case, focus on the point with the highest dilution (in this case 100×) because this sample contains the lowest level of contaminants.

! Please note that the calibration curve should cover concentrations of all dilutions of test samples, and the working range of the kit is 10 ng/mL to 10 µg/mL for cuvette fluorometers and 100 ng/mL to 10 µg/mL for plate fluorometers. However, avoid using too small volumes when diluting the initial sample because inaccuracy when pipetting small volumes can affect measurement results.

5. Incubate all the tubes (with protein standards and test samples) for 10 min at 90 to 95 °C in the dark.
6. Allow the contents of all tubes to cool down to room temperature (for about 15 to 20 minutes) without exposing them to light.
7. Discard the condensate by centrifugation.
8. Load 200 µL aliquots of the test samples and protein standards to 96-well plate wells.
9. **Fluorescence measurements.**

   Use a suitable source of excitation light and detection channel: ProteOrange reagent in the complex with a protein has an absorption maximum at ~485 nm and an emission maximum at ~590 nm.

10. Generate a calibration curve using fluorescence intensity values of protein standards. Using polynomial approximation, determine the equation of protein concentration (µg/mL) vs. fluorescence intensity (RFU) and calculate protein concentration in the test samples.

Fluorescence intensity of ProteOrange to BSA complex vs. BSA concentration. BSA concentrations are from 100 ng/mL to 10 µg/mL. Fluorescence measurement using plate fluorometer, optical filters for excitation and detection are 485/10 and 575/20 nm, respectively.

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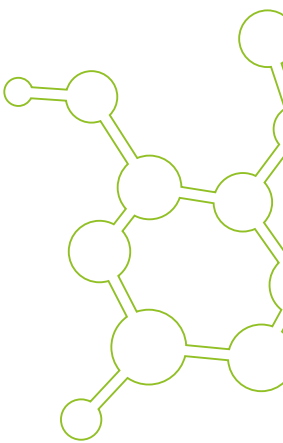

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