Protein Quantitation using the NanoOrange® Protein Quantitation Kit and the Quantus™ Fluorometer

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Materials Required

- NanoOrange® Protein Quantitation Kit (Life Technologies Cat.# N6666)
- Quantus™ Fluorometer (Cat.# E6150)
- 0.5ml PCR Tubes (Axygen Cat.# PCR-05-C, available through Fisher or VWR)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *Quantus™ Fluorometer Operating Manual* #TM396 is available at: **www.promega.com/protocols/**

The detection and quantitation of proteins in solution is a critical component of many biological applications. The use of fluorescent dyes, such as the NanoOrange® Protein Quantitation Kit, allows accurate protein detection with a level of sensitivity considerably better than that achieved with non-fluorescent methods such as the BCA method, Lowry assay, Bradford assay, or absorption at 280nm.

This Application Note describes the protocol for using the NanoOrange® Protein Quantitation kit with the Quantus™ Fluorometer (Cat.# E6150) to measure protein concentration. The protein sample is added to the diluted NanoOrange® reagent and the mixture heated at 95°C for ten minutes. Fluorescence is measured with the Quantus™ Fluorometer as soon as the mixture has cooled to room temperature. The NanoOrange® assay is designed to detect from 10ng/µl to 100pg/µl protein in a 200µl assay.

Protocol

1. Create a custom protocol on the Quantus™ Fluorometer by selecting "New" from the menu on the Protocol screen, and name the protocol using the up or down buttons. Enter the standard value of 10µg/ml. Select the Blue channel, and save the protocol.

   Note: The standard value was calculated by dividing the amount of BSA added to the standard (2mg) by the assay volume (200µl).

2. Equibrate all reagents to room temperature.
3. Dilute the concentrated NanoOrange® Protein Quantitation Diluent 10-fold in distilled water. For each assay, 200µl of 1X Protein Quantitation Diluent will be required.
4. Dilute the NanoOrange® Protein Quantitation Reagent 500-fold into the 1X Protein Quantitation Diluent. For example, add 10µl NanoOrange® Protein Quantitation Reagent to 4,990µl of 1X Protein Quantitation Diluent.

   Note: Protect the 1X NanoOrange® working solution and all samples from light at all times.

5. Prepare the BSA Standard by diluting the 2mg/ml BSA standard (Component B) 1:200 in 1X NanoOrange® working solution. Add 1µl of BSA standard to 199µl of working solution in a 0.5ml PCR tube. The Blank sample is 200µl of working solution.
6. Prepare the unknown samples by combining 1–8µl of sample with enough 1X NanoOrange® working solution to bring the final assay volume to 200µl.
7. Incubate all samples at 90–96°C for 10 minutes, protected from light.
8. Remove samples from the heat and let them cool to room temperature for at least 20 minutes, protected from light.
9. Select the custom protocol created in Step 1. Go to the Calibration screen and read the Standard and Blank samples prepared in Step 5. Save the calibration.
10. Enter the volume of the unknown samples and the desired concentration units.

    Note: This volume is the amount of sample that is added for the quantitation. For example, if 1µl of sample was added with 199µl of 1X NanoOrange® working solution, then the volume entered on this screen should be 1µl.
11. Place the unknown sample into the tube holder, and close the lid. The instrument will automatically measure fluorescence when the lid is closed, and the calculated concentration will be displayed.

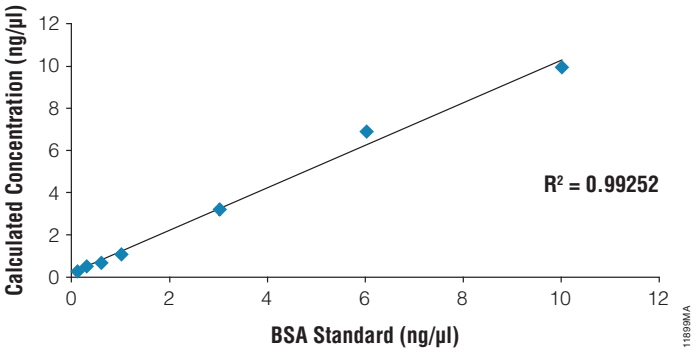

Figure 1. Measuring protein concentration using the NanoOrange® Protein Quantitation Kit and the Quantus™ Fluorometer. The standard curve was generated according to the manufacturer's instructions to demonstrate the linearity of the data generated using the Quantus™ Fluorometer. Samples were run in duplicate.