FluoroProfile® Protein Assay

Introduction

The FluoroProfile® protein dye is a fluorescent stain for quantitating minute amounts of protein in solution. Used in conjunction with the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer, the FluoroProfile assay provides a highly sensitive means of protein quantitation with minimal consumption of sample. The FluoroProfile protein dye consists of the natural product, eppicocconone. When activated with the blue LED (470 nm), the fluorescence of the protein-eppicocconone complex has an emission wavelength range of 550-700 nm with a maximum of ~614 nm. Unlike other protein assays, the bound protein complex is reversible and is recoverable for use in downstream applications such as mass spectroscopy and functional assays. The assay exhibits low protein-to-protein variation and works with many different types of proteins except those containing heme groups. Using as little as 2ul per measurement, the NanoDrop 3300 allows significantly reduced reaction volumes requiring a fraction of the total volume needed for conventional cuvette-based fluorometers.

Dynamic Range

The linear range of the FluoroProfile protein assay on the NanoDrop 3300 is between 3 ug/ml - 100 ug/ml. Larger ranges may require the use of a 3rd order polynomial curve fit while extended incubation times may enhance linearity at higher protein concentrations.

FluoroProfile Protein Assay Supplies

Equipment:

- NanoDrop 3300 Fluorspectrometer
- 2ul pipettor (low retention tips)

Materials:

- Low lint laboratory wipes
- Amber or foil covered 1.5ml polypropylene tubes or
- 0.2 ml strip tubes and caps

Reagents:

- FluoroProfile Protein assay kit from Sigma-Aldrich, which includes a dry stock BSA standard

Assay Recommendations

- Mix all solutions gently to avoid micro bubbles.
- Measure 2 ul sample aliquots.
- Remove samples from the optical surfaces by blotting rather than wiping to reduce residual lint fibers .
- Equilibrate all reagents, unknowns and protein standards to room temperature.
- Protect reagents from light.
- Mix both the concentrated FluoroProfile protein reagent and the buffer thoroughly.
- For additional information regarding the FluoroProfile Protein assay kit, refer to the the Sigma Aldrich website.

Protocol (3 ug/ml– 100 ug/ml Protein)

1. To prepare enough of the FluoroProfile protein working reagent for 50 samples, dilute the stock FluoroProfile protein reagent ten (10X) fold by transferring 25 ul of FluoroProfile protein buffer and 25 ul of the concentrated reagent to 200 ul of water in an amber tube. Mix gently by pipetting up and down to avoid introduction of air bubbles.

2. Mix each stock BSA standard solution thoroughly and transfer 5 ul of each BSA standard to the respective amber tube to obtain a total reaction volume of 10 ul .

3. Pipette 5 ul of the unknown samples to the appropriate amber tube containing 5 ul of the FluoroProfile proteinnworking solution.

4. Transfer 5 ul of the FluoroProfile protein working solution into amber tubes (one tube per each standard and unknown sample).

5. Mix each standard and unknown sample thoroughly, collect the solution at the bottom of the tube by a brief centrifugation, and allow the reaction to incubate at room temperature for 30 minutes. The final reaction volume for each standard and unknown should be 10 ul.

Note: The assay is stable for up to 6 hours but it is recommended the samples be measured immediately after the standard curve has been established.

6. Mix well, collect the solution at the bottom of the tube by a brief centrifugation, and proceed to the NanoDrop 3300 standard curve protocol on page 2.

Standard Curve Protocol

1. Clean both sampling pedestals with 2 uL of nuclease free deionized water.

2. Open upper arm and firmly blot the two pedestals with a dry lab wipe. Make sure there are no traces of lint on the pedestals before continuing.

3. Open the operating software. Click on the Protein Quantitation button and select the FluoroProfile method.

4. Add 2 uL of assay buffer (no dye, no sample) to the lower pedestal. Lower the arm and click F3 or the Blank button. When the measurement is complete, lift the arm and use a dry laboratory wipe to blot the buffer from both the bottom and upper measurement surfaces. Use a fresh aliquot of buffer to verify a proper baseline.

5. Under Measurement type, click on the Standards tab. Highlight the Reference standard.

6. Mix the reference solution (assay buffer and dye, no sample) briefly and transfer 2 uL of the solution onto the lower pedestal. Lower the arm and click F1 or the Measure button. A pop up window will ask for confirmation of the units. (Recommended ng/mL or pg/uL)

7. Measure up to 5 replicates of the reference solution using a fresh 2 uL aliquot for each measurement.

8. Select Standard 1 to enter a value. Enter values for up to 7 standards.

9. Mix the standard solution briefly and transfer 2 uL onto the lower pedestal. Lower the arm and click F1 or the Measure button. Measure up to 5 replicates of each standard using a fresh 2 uL aliquot for each measurement.

10. Once the standard curve is completed, select the Standard Curve Type (Interpolation, Linear, 2° polynomial, 3° polynomial) that best fits the standards data set.

11. Click on the Sample tab under Measurement Type, and enter the unknown samples' respective ID information. If a dilution of the unknown sample was made, enter the dilution factor in the box below the sample ID window.

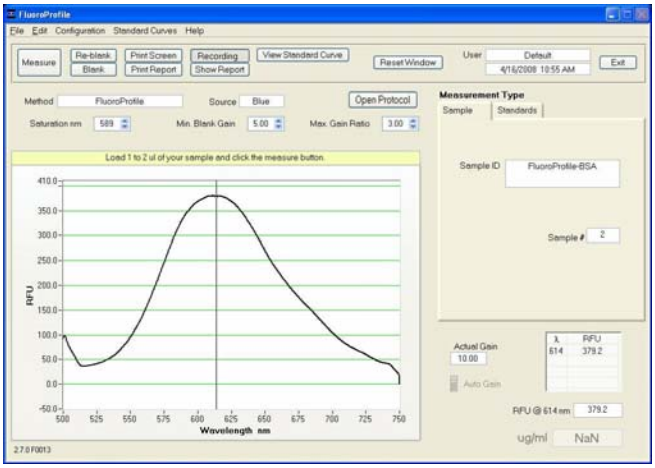
12. Add 2 ul of the sample and use the F1 key or click the Measure button to initiate the measurement cycle. Use a fresh aliquot of sample for each measurement.

Performance Data

Table 1. Typical data for the FluoroProfile protein assay using the Blue LED and measured at an analysis wavelength of 614 nm.

BSA (ug/ml)	Ave RFU (n=4)	St dev	%CV
0	41	1.7	4.2
1.56	48	2	4.2
3.125	60	1.4	2.3
6.25	88	1.3	1.5
12.5	179	7.6	4.2
25	384	6.3	1.6
50	765	10.5	1.6
100	1502	26	1.7

Example spectrum of FluoroProfile protein sample.



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