Fluoraldehyde ™ OPA Protein Assay

Introduction

The Fluoraldehyde™ o-Phthaladehyde,(OPA), reagent is a ready-to-use formulation of OPA that offers a rapid fluorescent detection of minute amounts of proteins and peptides in solution. Used in conjunction with the micro-volume capability of the Thermo Scientific NanoDrop™ 3300 Fluorospectrometer, the Fluoraldehyde™ reagent provides a highly sensitive means of protein quantitation with minimal consumption of sample. The ability of the NanoDrop 3300 to measure as little as 2 ul of protein samples allows significantly scaled-down reaction volumes, thereby using only a fraction of sample and reagent commonly needed for conventional cuvette-based fluorometers. **Notes:** there is considerable protein-to-protein and peptide-to-peptide variation with this assay so use of a purified sample of the particular protein or peptide as a standard is recommended. Buffers containing primary amines are not recommended. Peptides or proteins with blocked primary amines will not give a response with OPA.

Dynamic Range

A wide dynamic range for the assay can be obtained by utilizing two distinct sample to reagent volumes. The high 15 ug/ml to 1000ug/ml range for BSA is covered by mixing 2 ul of protein sample with 20 ul of reagent. The sensitivity of the assay may be increased to 1-50 ug/ml by using a higher sample volume to reagent volume.

High Range (15 ug/ml–1000 ug/ml)

A 1 to 10 sample to reagent volume is typically used for a high range assay.

Low range (1 ug/ml– 50 ug/ml)

A 1 to 0.5 sample to reagent volume is typically used for a low range assay.

Supplies

Equipment:

- NanoDrop 3300 Fluorospectrometer
- 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:

- Fluoraldehyde™ Reagent, Pierce Cat. # 26025
- Pierce BSA standard cat # 23209 (or other protein/peptide standard)

Assay Recommendations

- Measure 2 ul sample aliquots.
- Mix all solutions gently to avoid micro bubbles.
- Remove samples from the optical surfaces by blotting rather then wiping to reduce residual lint fibers from collecting on the NanoDrop 3300 sampling pedestals.
- Equilibrate all reagents, unknowns and protein standards to room temperature.
- Protect the reagent and solutions containing reagent from light.
- For additional information regarding the Fluoraldehyde™ reagent, please refer to the Pierce web site.

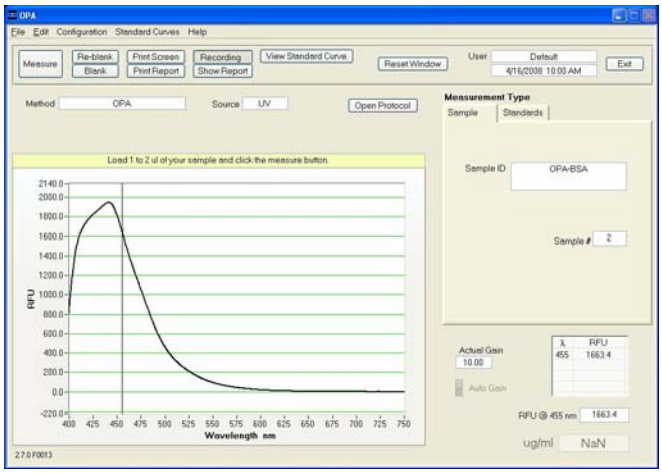
Fluoraldehyde ™ OPA Protein Assay

1. Mix each stock standard solution and unknown samples thoroughly and transfer 10 ul (low range) or 2 ul (high range) of each to the respective amber tubes.

2. Add 5 ul (low range) or 20 ul (high range) of the Fluoraldehyde™ reagent into each standard and sample and mix each standard and unknown sample thoroughly by pipetting up and down several times. Optonal: collect the solution at the bottom of the tube by a brief centrifugation,

3. Allow the reaction to incubate 10 minutes at room temperature. Note: It is recommended that the samples be measured immediately after the standard curve has been established.

Example spectrum of Fluoraldehyde ™ OPA Protein sample



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Standard Curve Protocol

1. Clean both sampling pedestals with 2 uL of nuclease free de-ionized 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 Flouraldehyde OPA 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.

Low Range (1 ug/ml–50 ug/ml) Dilution Series

BSA (ug/ml)	Ave RFU (n=5)	St dev	%CV
0	199	8.4	21
0.8	380	12	14
1.6	630	35	29
3.1	1305	19	6
6.3	2462	50	6.7
12.5	4858	31	1.9
25	8523	32	1
50	14863	79	1.4

High Range (15 ug/ml–1000 ug/ml) Dilution Series

BSA (ug/ml)	Ave RFU (n=5)	St dev	%CV
0	199	56	28
16	16	75	20
31	31	48	7.7
63	63	22	1.7
125	125	162	6.7
250	250	150	3
500	500	79	0.9
1000	1000	256	1.7