SensoLyte™ OPA Protein Quantitation Kit **Fluorimetric**

Revision# 1.2	*Last Updated: July 2021*
Catalog#	**AS-71015**
Kit Size	500 Assays (96-well plate)

- ***Optimized Performance:*** Optimal conditions for protein detection.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	OPA	350 µL
Component B	Reducing solution	200 µL
Component C	Assay buffer	20 mL
Component D	BSA standard	1 mg/mL, 500 µL

Other Materials Required (but not provided)

- 96-well microplate or cuvette: black microplate or quarts cuvettes.
- Fluorescence microplate reader or fluorometer: Capable of detecting emission at 440-480 nm with excitation at 335-345 nm.

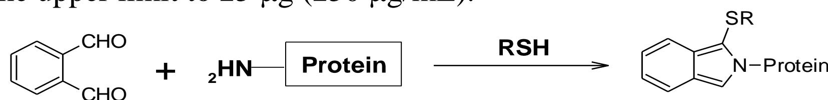
Storage and Handling

- Store all kit components at -20°C.

Introduction

o-Phthalaldehyde (OPA) in the presence of a reducing reagent will react with α-amino acid to form an intense blue fluorescent product (**Scheme 1**). The fluorescence can be monitored at Ex/Em=338±5 nm/455±10 nm. It has been used in column chromatographic amino acid analysis since 1970s[1]. It can precisely detect nmole level of amino acid and is at least 50-100 fold more sensitive than its colorimetric counterparts, ninhydrin[1].

AnaLyte™ OPA Protein Quantitation Kit employs the OPA for rapid and sensitive protein quantitation in solution. A proprietary odorless reducing reagent is used in the assay formula to replace 2-mercaptoethanol, which has unpleasant odor. The kit functions well in the presence of lipids, detergents, and reducing reagent (such as DTT), the substances that interfere with many other protein determination methods. The assay can detect as low as 0.3 µg (3 µg/mL) of protein with the upper limit to 25 µg (250 µg/mL).



Scheme 1. The fluorogenic reaction of OPA with proteins in the presence of a thiol compound

Protocol

Note: For standard curve, please refer to Appendix I (optional).

1. **Prepare protein standard.**

   1.1 Bovine serum albumin (BSA) standard: Add 100 µL of BSA standard (Component D, 1 mg/mL) to 900 µL assay buffer (Component C) or phosphate-buffered saline to get a concentration of 100 µg/mL. Then do a series of two-fold dilutions in assay buffer (Component C) or phosphate-buffered saline to get the concentrations of 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8 µg/mL. Prepare a blank control, which contains assay buffer or phosphate-buffered saline but no BSA.

   Note: The fluorescence of different protein-OPA complex varies dramatically. Ideally, you should use the same protein in your test samples as standard.

2. **Prepare protein samples.**

   2.1 If the protein sample is powder, dissolve it in assay buffer (Component C) or phosphate buffered saline. If the protein sample is in high concentration, dilute it with assay buffer (Component C) or phosphate buffered saline. If protein samples are prepared in buffers other than assay buffer (Component C) or phosphate buffered saline, set up a control for this buffer. You may prepare three to four different dilutions of your protein samples for the assay, so that at least the concentration of one dilution will fall into the concentration range of standard curve.

   Note: Avoid ammonium ions and amine-containing buffer, e.g., Tris and glycine, when preparing your protein samples, since amine can react with OPA to generate fluorescence background.

   2.2 Add 100 µL/well of protein samples, blank control, buffer control, and BSA standards to a black microplate.

3. Prepare OPA assay solution according to table 1. Mix the reagent well.

Table 1. OPA assay solution for one 96-well plate (100 assays).

Components	Volume
OPA (Component A)	60 µL
Reducing solution (Component B)	24 µL
Assay buffer (Component C)	1.916mL
Total volume	2 mL

Note: The OPA assay solution should be prepared freshly for each experiment.

4. Start the protein assay.

4.1 Add 20 µL/well of OPA assay solution to the BSA standard, blank control, buffer control, and test sample wells. Cover the plate with aluminum foil. Incubate the plate on a plate shaker at 100-200 rpm for 1-2 hr at room temperature. The reaction can be prolonged to 5 hr. All of the samples and standards should have the same reaction time for better comparison.

4.2 Measure fluorescence at Ex/Em=338±5 nm/455±10 nm.

Note: For cuvette assay that requires the total volume larger than 100 µL, you may dilute the final reaction mixture with assay buffer (Component C) before measuring the fluorescence.

5. Data analysis.

- The fluorescence reading from the blank control is the background fluorescence. Subtract this background reading from the readings of the other wells to get relative fluorescence unit (RFU) for all the samples.
- Plot the BSA standard as fluorescence unit (RFU) versus concentration (Figure 1).

  Note: The BSA standard curve is used to calculate the protein concentration of your samples. The BSA standard curve is also used to calibrate for the variation of different instruments and for different batches of experiments.
- Calculate the protein concentration of the samples according to the BSA standard curve.

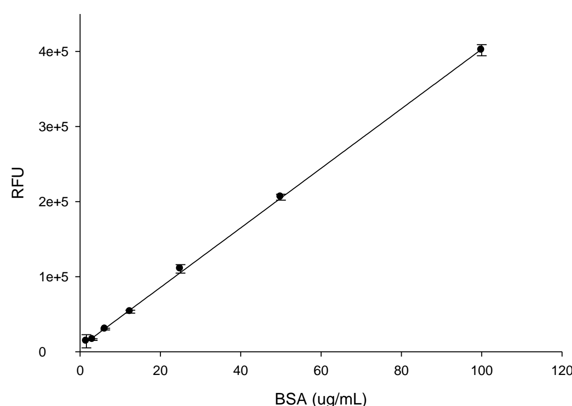


Figure 1. The detection range of OPA protein assay.

BSA is serially diluted in phosphate buffered saline. 100 µL of BSA serial dilutions were mixed with 20 µL of OPA assay solution, and then incubated for 1 hr in dark. The fluorescence signal was detected at Ex/Em=338/455 nm, cut off 435 nm by Flexstation 384II, Molecular Devices. (n=3, mean±S.D.)

Reference :

1. Roth, M. et al. *J Chromatogr* **83**, 353 (1973).