FT-51225A

OPA Protein quantitation kit

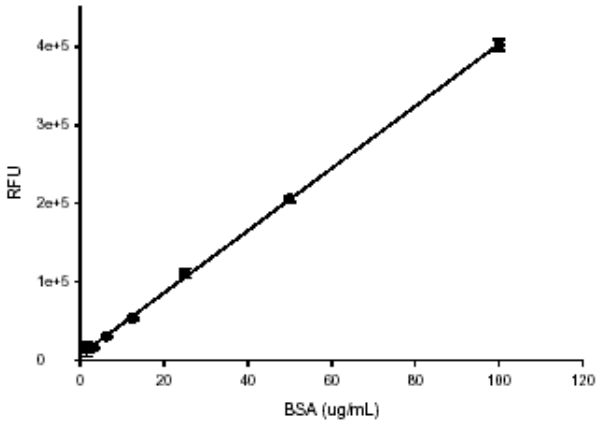
A sensitive fluorigenic protein Protein Assay

Description

Catalog #: 51225A, 1 kit (500tests)

Name: **OPA Protein Quantitation**

Contains: reagent A: OPA reagent (350µl)
reagent B: Reducing solution (200µl)
reagent C: Assay buffer (20ml)
reagent D: BSA standard 1mg/ml (500µl)
(bovine serum albumin)



0.1µg/ml – 50µg/ml working range

Figure 1. 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. (n=3, mean±S.D.)

Storage: -20°C (M) (+4°C for short term)

Applications: Protein Quantitation in various applications, including especially:

- for higher sensitivity than conventional, and/or better compatibility with other substances
- before protein analysis (SDS-PAGE, Capillary electrophoresis, Immunoassays,...)
- after protein extraction (interfering detergents)

AA & Peptides Quantitation
HPLC post column derivatization

Introduction

This kit is designed to quantify proteins as well as peptides (even small ones) in solution in the range from 0.1µg/ml to 50µg/ml. It provides sufficient materials to perform 500 assays in a 96-wells format microplates, or twice in 384-wells. It also can be used to detect or quantitate amino-acids, i.e. in chromatography.
It uses OPA reagent for rapid and sensitive protein detection. Unlike home-made protocols and other OPA based kits, it is odorless thanks a proprietary formulation. The kit functions well in presence of lipids, reducing agents and detergents. The OPA-based fluorometric protein assay functions well in the presence of lipids and detergents, substances that interfere with many other protein determination methods. It gives faster and more sensitive detection especially of peptides that other methods. However it not suit acetylated and other primary amine-blocked peptides.

Directions for use

Protocol 1: standard protein/peptide assay

1- Allow all reagents to reach room temperature before use.

2- **Prepare protein standard (BSA) standard**:

Add 100 µL of BSA standard (reagent D, 1 mg/mL) to 900µL assay buffer (reagent C) to get a concentration of 100 µg/mL.
Then do a series of two fold dilutions in assay buffer (C) : concentrations of 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, 0.4, 0.2, 0.1 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, and may be slightly affected by the sample buffer. Ideally, you should use as standard thus the same protein in your test samples, prepared in Assay buffer, or dilute de BSA standard in the sample buffer, i.e. phosphate-buffered saline.

3- **Prepare protein samples at suitable dilution**.

• If the protein sample is powder, dissolve it in assay buffer (C) or phosphate buffered saline. If the protein sample is in high concentration, dilute it with assay buffer (C) or phosphate buffered saline.
• If protein samples are prepared in buffers other than assay buffer (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.

4- **Add 100 µL/well of protein samples**, blank control, buffer control, BSA standards to a black microplates (FPlyte, see related products).

5- **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 (reagent A)	60 µL
Reducing solution (reagent B)	24 µL
Assay buffer (reagent C)	**2 mL**
Total volume	*2 mL*

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

6- **Start the protein assay**:

• 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 100-200rpm 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.

• **Note:** For a 384-well plate, add 50 µL sample and 10 µL OPA assay solution per well.

• 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 (C) before measuring the fluorescence.

7- **Data analysis**:

• Subtract the background (fluorescence reading from the blank control) 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.

Technical and Scientific Information

Principle

The kit employs o-Phthalaldehyde (OPA) in the presence of a reducing reagent, that reacts with α-amino acid to form a intense blue fluorescent product (scheme 1). The fluorescence can be monitored at Ex/Em=338±5 nm/455±10 nm. OPA was popularized in column chromatographic amino acid analysis as it can precisely detect nmol levels of amino acid and is at least 50-100 fold more sensitive than its colorimetric counterpart, ninhydrin [1]. Homemade protocols and other OPA based kits perform the condensation reaction of OPA with amino-containing compounds requires the coexistence of thiol compound (such as 2-mercaptoethanol) that usually has strong unpleasant odor. Now, our kit use a proprietary odorless reducing reagent.

Compatibility & applications

Our OPA Quantitation Kit detects efficiently proteins, as well as peptides and amino-acids that are not detected by colorimetric methods such as Bradford and Lowry methods. The assay can detect as low as 0.3 µg/mL of protein with linear signal up to 50 µg/mL. It functions well in the presence of lipids, detergents, and reducing reagent (such as DTT), the substances that interfere with many other protein determination.

This makes it useful in applications such as:

- when standard protein dosage - do not achieve sufficient sensitivity of detection (a)
  - is not compatible with (interfering) substances (b)
- (a) i.e. samples prepared for SDS-PAGE electrophoresis (SDS and DTT or b-mercatoethanol interferences)
  samples prepared for immunoassays (interference of detergents such as Tween20, Tween80...)
  protein of tissues, cells and bacteria extracts (interference of detergents such as TritonX100, Brij35 or CHAPS).
- (b) i.e. peptides quantitation. However, the kit does not suit acetylated and or other amine-blocked peptides.

Reference:

Roth, M. and A. Hampai, J.Chromatogr. 83, 353-356 (1973).

Other Information

Related / associated products

FPlyte microplates (microplate optimized for fluorescence)
i.e. 96-wells with clear bottom – No cross-talk – with white (#FP-BA7950) or black (#FP-BA7990) matrice and walls.
OPA powder #FP-02727B (for homemade protocols)
BC Assay #UP40840A (colorimetric assay, with much larger working range)
Coo Assay #UPF4600 (colorimetric assay, quicker)
For in vitro R&D use only
Please contact Uptima – Interchim for any other information

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