Product Manual

High Sensitivity Protein Quantitation Assay Kit (Fluorometric)

Catalog Number

AKR-185 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Protein quantitation of aqueous samples is a fundamental assay performed in most biology labs; an essential tool during protein purification, chromatography, recombinant protein expression, peptide synthesis, etc. Fluorescence-based protein determination methods provide the highest sensitivity when compared to standard colorimetric assays (Biuret, Lowry, BCA, and Bradford).

Cell Biolabs' High Sensitivity Protein Quantitation Assay Kit utilizes a fluorogenic detection dye to measure primary aliphatic amines (peptides or proteins) in various samples with much greater sensitivity than the conventional colorimetric protein determination methods. The assay is performed in a 96-well microtiter plate format and measured within 15 minutes. Each kit provides sufficient reagents to perform up to 200 assays, including blanks, standards and unknown samples. The kit contains a BSA standard and has a detection sensitivity limit of < 5 µg/mL.

Kit Components

1. BSA Protein Standard (Part No. 218501): One 1 mL vial of 10 mg/mL BSA (sterile filtered).
2. Protein Detection Dye (Part No. 218502): Four 1.3 mL vials.

Materials Not Supplied

1. PBS
2. 96-well fluorescence black microtiter plate
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Fluorescence microplate reader capable of reading excitation in the 355-385 nm range and emission in the 460-470 nm range.

Storage

Store the entire kit at -80ºC. Avoid multiple freeze/thaws by aliquoting. The Protein Detection Dye is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- Components should be thawed/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.

Preparation of BSA Standard

- Freshly prepare a dilution series of the standard in the concentration range of 0 µg/mL – 500 µg/mL by diluting the standard stock solution (provided at 10 mg/mL) in 1X PBS (see Table 1). If another buffer is desired, see the Potential Interference and Compatibility Section below. BSA standards should be prepared fresh.

Standard Tubes	10 mg/mL BSA Standard (µL)	1X PBS or Desired Buffer (µL)	Final BSA Standard (µg/mL)
1	50	950	500
2	500 of Tube #1	500	250
3	500 of Tube #2	500	125
4	500 of Tube #3	500	62.5
5	500 of Tube #4	500	31.25
6	500 of Tube #5	500	15.63
7	500 of Tube #6	500	7.81
8	0	500	0

Table 1. Preparation of BSA Standards

Potential Interference and Compatibility

Buffers containing primary amines are known to interfere with the assay and should be avoided. Table 2 contains some common substance compatibilities. However, to accurately determine the degree of compatibility by a substance/buffer, the BSA standard curve should be prepared in PBS vs. sample substance/buffer. Dilution of the substance/buffer, and ultimately samples, may be required to completely eliminate interference. Even with some interference, accurate quantitation can be achieved by running standards in the same buffer as samples, although kit sensitivity may be compromised.

Substance	Compatible Concentration*
Acetone	5 %
Deoxycholic acid	N/C
Dithiothreitol	1 mM
DMEM with phenol red	N/C
EDTA	N/C
Ethanol	10 %
Glutathione	N/C
Glycerol	10 %
Glycine	N/C
Hepes, pH 7.5	50 mM

Substance	Compatible Concentration*
Isopropanol	10 %
2-Mercaptoethanol	1 mM
Methanol	10 %
NP-40	10 %
PBS	undiluted
Sodium Chloride	1 M
SDS	N/C
Tris, pH 7.5	N/C
Triton-X 100	1 %
Tween-20	1 %

*N/C = Not compatible with this assay

Table 2. Substance Compatibilities

Assay Protocol

Each BSA standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 75 µL of the diluted BSA standards or samples to the 96-well fluorescence microtiter plate.
2. Add 25 µL of Protein Detection Dye to each well.
3. Cover the plate wells to protect the reaction from light.

4. Incubate at room temperature for 15 minutes on an orbital shaker. Incubations may be shortened down to 5 minutes, but sensitivity may be reduced.
5. Read the plate with a fluorescence microplate reader equipped for excitation in the 355-385 nm range and for emission in the 460-470 nm range.
6. Calculate the concentration of protein within samples by comparing the sample fluorescence to the standard curve. Negative controls (without BSA) should be subtracted.

Example of Results

The following figures demonstrate typical Protein Quantitation Assay Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

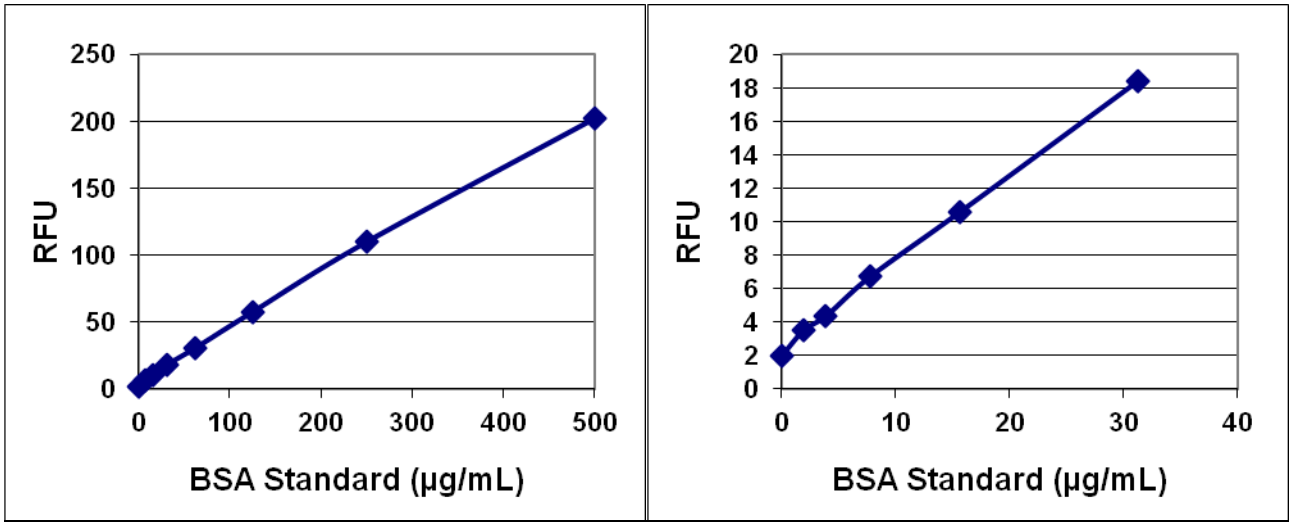

Figure 1: High Sensitivity Protein Quantitation Assay Standard Curve. BSA standard curve was generated according to the Assay Protocol.

References

1. Eggenreich, K., Zach, E., Beck, H., and Wintersteiger, R. (2004) *J. Biochem. Biophys. Methods* **61**, 35.
2. Adamou, R., Coly, A. Douabale, S.E., Saleck, M.L., Gaye-Seye, M.D., and Tine, A. (2005) *J. Fluoresc.* **15**, 679.
3. Eastwood, D., Fernandez, C., Yoon, B.Y., Sheaff, C.N., and Wai, C.M. (2006) *Appl. Spectrosc.* **60**, 958.

Warranty

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