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FOR *IN VITRO* USE ONLY.

Protein Assay ESL

Exact, Sensitive, Low interference
For determination of protein concentrations in solution

Cat. No. 1 767 283 125 assays
Cat. No. 1 767 003 500 assays

Version 3, November 1999

Store at 2-8° C

Product description

Protein Assay ESL is a kit which allows for spectrophotometric determination of protein concentrations. This method is an optimized procedure derived from (1) and utilizes a biuret-like reaction, whereby Cu^{2+} initially complexes with protein in alkaline medium.
Protein content is then determined by measuring the absorbance of a Cu^{+}-bathocuproine complex which is formed with excess Cu^{2+} not chelated by protein.
Excess Cu^{2+} is reduced to Cu^{+} with ascorbic acid which can then form a colored complex with bathocuproine. The signal is inversely related to the amount of **peptide bonds**, in contrast to other common protein assays which are influenced by specific amino acid side chains. Therefore, protein-to-protein variability is very low and individual proteins or protein mixtures can be measured accurately.

The primary advantages of this method include:

- far less protein to protein variability as compared to other protein assays,
- assay speed,
- good reagent stability,
- excellent sensitivity,
- broad linear range,
- broad tolerance for potentially interfering substances.

Stability

All solutions are stable until the date stated on the label when stored at 2-8° C in tightly sealed bottles (all solutions) and protected from light (Reagent B).
To ensure longer stability Reagent A and Reagent B have been aliquoted and bovine serum albumin (BSA) protein standard solution is stabilized with an antimicrobial agent.

Kit content

Protein Assay ESL contains the following items:

- 4 × 13 ml Reagent A
  (alkaline copper tartrate solution)
- 4 × 130 ml Reagent B
  (ascorbic acid, bathocuproine disulfonic acid solution)
- 1 × 2 ml BSA protein standard (2 mg/ml)
  in 0.9% NaCl, stabilized.

Additionally required materials

- Pipettes 50 µl, 100 µl, and 1000 µl
- disposable 1.5 ml cuvettes
- spectrophotometer capable of measuring at 485 nm

Sensitivity and linear range

Protein Assay ESL can accurately detect 20 µg protein/ml in solution and has a linear measurement range of 20–800 µg/ml.

Applications

Assay time

Using Protein Assay ESL a sample can be prepared and measured in only 7 minutes.

Additional advantages

- **Small sample amount**
  with Protein Assay ESL only 50 µl of sample is required (standard assay procedure). The detection limit (absolute amount) is then 1 µg/sample.
- **Automation**
  Protein Assay ESL can readily be adapted to a microtiter plate format.

Standard assay procedure

1. Allow Reagent A and Reagent B to warm to 15-25° C or place in a 37° C water bath.
2. Prepare a series of standards covering an appropriate concentration range by diluting the BSA protein standard in the same diluent as the unknown sample.

Tube no.	Diluent*(µl)	BSA protein standard (2 mg/ml)	Final concentration (µg/ml)
1	400	0	0
2	390	10	50
3	380	20	100
4	340	60	300
5	300	100	500
6	250	150	750

*Unless it is known that a diluent does not interfere with the assay, it is important to prepare the standards in the same matrix in which the unknown is present.

3. Add 100 µl Reagent A to the appropriate number of semi-micro (1.5 ml) cuvettes.
4. Add 50 µl of standard or sample to the appropriate cuvette and mix.
5. Incubate cuvette at 15-25° C for at least 5 min. Prolonged incubation with Reagent A (up to 1 h) does not affect assay performance.
6. 6. Add 1000 µl Reagent B to the first cuvette, mix briefly, and measure absorbance at 485 nm against a water or diluent reference cuvette after **exactly** 30 s
7. Repeat step 6 sequentially with the remaining samples.
8. Construct a calibration curve by plotting absorbance of the standards against their concentration. From this calibration curve, the protein concentration of the unknown sample can be calculated.

For repetitive measurements of a sample in the same diluent, it is normally sufficient to construct a calibration curve once, and thereafter validating the calibration curve with absorbance obtained for the zero point (tube no. 1 in table above).

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Reagent compatibility

The following biochemical reagents were tested in conjunction with Protein Assay ESL. A series of BSA protein standards (50–750 µg/ml) were made containing the listed compounds at the indicated concentrations and were found not to interfere with the assay. For measuring protein concentrations in solutions containing compounds not included in this list, it is necessary to make a calibration curve using calibration standards prepared in the same buffer as the sample of interest.

	Compound	Concentration
Detergents	Octylglucoside	1 % (w/v)
	Triton[1)]X-100	0.5 % (w/v)*
	Brij[2)]35	0.5 % (w/v)*
	Thesit[3)]	0.5 % (w/v)*
	Nonidet[4)]P40	0.5 % (w/v)*
	CHAPS	0.5 % (w/v)*
Buffers	Hepes	0.5 M, pH 5.1*
	Sodium phosphate	0.5 M, pH 7.5*
	Glycine	0.2 M, pH 2.8
Miscellaneous	Ammonium acetate	0.5 M*
	Micr-O-protect[5)]	0.1 % (v/v)*
	$CaCl_2$	5 mM*
	DTT	0.2 mM
	Sodium azide	0.1 % (w/v)

* The assay can be performed in the presence of these compounds, however a separate calibration curve is required.

Protein to protein variability

Most widely used protein assays exhibit strong protein-dependent responses and are therefore generally useful for providing only relative protein concentrations (2,3,4). This is not the case with Protein Assay ESL which shows minimal protein to protein variability, as shown in the figure below. IgG, cytochrome C and BSA, proteins known to respond quite differently in other protein assays, were assayed with Protein Assay ESL, whereby only minimal variability was demonstrated. Each data point in the figure is the average value of measurements, made in triplicate, and the solid lines represent linear regressions of these data.

Additional applications

Peptides

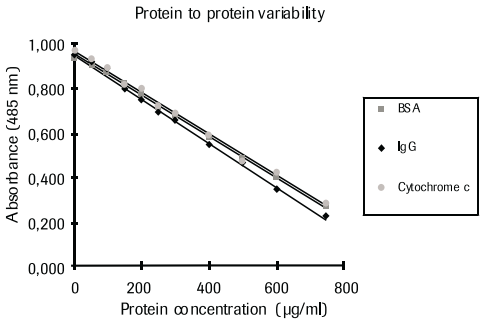
Initial experiments have shown that Protein Assay ESL can also be used to determine peptide concentrations. Peptide concentrations are determined using the same procedure described above.

Immobilized proteins

Protein Assay ESL has been used successfully for determining protein bound to a solid phase matrix, such as agarose. For this application, the standard assay procedure is modified slightly. In step 4, the 50 µl sample is replaced with a pre-weighed amount of matrix-bound protein. All further steps remain the same.

Quality control

Each lot and all components of Protein Assay ESL are function-tested to ensure the specified performance. When stored as indicated, Protein Assay ESL will function properly through the expiration date.



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2 Lowry, O.H., Rosebrough, N.J., Farr, A L & Randall, R.J. (1951) *J. Biol. Chem.* **193**, 265–275.
3 Bradford, M.M. (1976) *Anal. Biochem.* **72**, 248–254.
4 Smith, P.K.et al (1985) *Anal. Biochem*. **150**, 76–85.

* available from Roche Molecular Biochemicals
1) Triton is a trademark of Rohm & Haas Company, Philadelphia, PA, USA.
2) Brij 35 is a trademark of ICI Americas, Inc., USA.
3) Thesit is a trademark of Desitin-Werk, Carl Klinke GmbH, Hamburg, Germany.
4) Nonidet is a trademark of Shell International Petroleum Company Limited, U.K.
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