WelProt™ Protein Assay Kit

Catalog Number **ML 050-01**
Storage Temperature Room Temperature (4°C for BSA)
Kit Content:

Protein Assay Reagent 1: 250 ml X 4
Protein Assay Reagent 2: 25 ml X 1
BSA Standard (2 mg/ml): 1 ml X 5
(For 500 Standard Assays / For 5000 Micro Assays)

Product Description

The protein quantification method using bicinchoninic acid was developed by Smith *et al*. (1). The WelProt™ Protein Assay Kit was based on this method. This kit detects the cuprous ions generated from cupric ions by reaction with protein under alkaline conditions. The Lowry assay and this assay are of similar sensitivity, but this assay is stable under alkali conditions and can be carried out as a one-step process compared to the two steps needed in the Lowry assay. The reaction results in the development of an intense purple color with an absorbance maximum at 562 nm. Since the production of Cu^{+} in this assay is a function of protein concentration and incubation time, the protein content of unknown samples may be determined spectrophotometrically by comparison with known protein standards. A further advantage of this assay is that it is generally more tolerant to the presence of compounds that interfere with the Lowry assay. In particular, it is not affected by a range of detergents and denaturing agents such as urea and guanidium chloride.

Storage/Stability

Store Reagent 1 and 2 at room temperature and BSA standards at 4°C. If WelProt™ Protein Assay Reagent 1 or 2 precipitates upon shipping or during long-term storage, dissolve precipitates by gently warming and stirring solution. Discard any kit reagent that shows discoloration or evidence of microbial contamination.

Chemical Hazard

If above solutions come into contact with eyes or skin, flush with plenty of water and remove contaminated clothing.

Protocol

Preparation of BSA Standards

Each 1 ml tube of 2 mg/ml BSA is sufficient to prepare a set of diluted standards for either working range suggested in Table 1.

Tube No.	Volume of diluent (µl)	Volume of BSA (µl)	BSA Conc. (µg/ml)
1	0	300, Stock Solution	2000
2	75	225, Stock Solution	1500
3	150	150, Stock Solution	1000
4	187.5	112.5, Stock Solution	750
5	438.75	146.25, Stock Solution	500
6	195	195, Tube No. 5	250
7	225	75, Tube No. 5	125
8	360	90, Tube No. 5	100
9	210	90, Tube No. 6	75
10	225	225, Tube No. 8	50
11	150	150, Tube No. 10	25
12	300	0	0

Table 1. Preparation of diluted BSA Standards

Preparation of Working solution

Mixing 50 parts of WelProtTM Protein Assay Reagent 1 and 1 part of WelProt™ Protein Assay Reagent 2 (The Working solution is apple green in color and is stable for 24 hours when stored in dark at room temperatue).

Standard assay (using test tube)

1. Add 100 µl of each standard and unknown sample replicate into test tubes.
2. Add 2 ml of the Working solution to each tube and mix well.
3. Incubate tubes at 37°C or 60°C for 30 min (37oC for 30 min : 0.1~2 mg/ml total proteins, 60°C for 30 min : 10~250 µg/ml).
4. Cool tubes to room temperature, then measure at 562 nm (see Note 1 and 2).
5. A calibration curve can be constructed using dilutes of BSA stock solution.

Micro assay (using microplate)

1. Add 10~25 µl of each standard and unknown sample replicate into a microplate.
2. Add 200 µl of the Working solution to each tube and mix well.
3. Incubate tubes at 37°C or 60°C for 30 min.
4. Cool tubes to room temperature, then measure at 562 nm.
5. A calibration curve can be constructed using dilutes of BSA stock solution.

Notes

1. All glassware must be cleaned and given a thorough final rinse with ultra pure water. Exercise care when re-using glassware.
2. Following the heating step, the developed color is stable for at least 1 hr.
3. Note, that like the Lowry assay, response to the assay using bicinchoninic acid is dependent on the amino acid composition of the protein, and therefore an absolute concentration of protein cannot be determined. The BSA standard curve can only be used to compare the relative protein concentration of similar protein solutions.
4. Because some reagents interfere with the bicinchoninic acid reaction, avoid the following substances as components of the sample buffer. If not removed from the assay, consider a different protein assay (e.g. Bradford assay) (2). Also the presence of lipids gives excessively high absorbance with this assay (3).

Ascorbic acid	EGTA	Iron
Creatinine	Hydrogen peroxide	Tryptophan
Cysteine	Phenol Red	Uric acid
Tyrosine	Impure Sucrose	Impure Glycerol

5. Since the method relies on the use of $Cu2^{+}$, the presence of chelating agents such as EDTA will of course severely interfere with the method. However, it may be possible to overcome such problems by diluting the sample as long as the protein concentration remains sufficiently high to be measurable.

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Troubleshooting

Problems	Comments and Suggestions
No color in any tubes	
Sample contains a copper chelating agent	Dialyze, desalt, or dilute sample Increase copper concentration in working reagent
Blank absorbance is OK, but standards and samples show less color than expected	
Strong acid or alkaline buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
Color measured at the wrong wavelength	Measure the absorbance at 562 nm
Color of samples appears darker than expected	
Protein concentration is too high	Dilute sample
Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids
All tubes including blank are dark purple	
Buffer contains a reducing agent, thiol, or biogenic amines	Dialyze or dilute sample
Need to measure color at a different wavelength	
Spectrophotometer or plate reader does not have 562 nm	Color may be measure at any wavelength between 540 nm and 590 nm, although the slope of standard curve and overall assay sensitivity will be reduced

References

Smith, PK, Krohn, RI, Hermansen, GT, Malia, AK, Gartner, FH, Provenzano, MD, Fujimoto, EK, Goeke, NM, Olson, BJ and Klenk, DC, Anal Biochem 150:76-85 (1985).
Brown, R, Jarvis, K and Hyland, K, Anal Biochem 180:136-9 (1989).
Kessler, RJ and Fanestil, DD, Anal Biochem 159:138-142 (1986).

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