57697 Advanced Protein Assay Reagent

Application

The Advanced Protein Assay Reagent is designed to optimize the speed and accuracy of protein measurement. The reagent combines the useful properties of low protein to protein variance and a strong signal for a sensitive assay. A simple one step procedure results in a green to blue color change which can be recognized by measuring absorbance at 600nm. It may be used for protein determination in buffers, biological fluids, or tissue culture media.

Content

1. Advanced Protein Assay Reagent (1 x solution) (100 or 500 ml)
2. Manual.

Equipment required

1. Spectrophotometer with 600nm wavelength.
2. Small capacity cuvettes (1.0ml) or 96 well plates with at least 300ul wells.
3. Pipettors 20ul, 1000ul and 5.0ml capacity.
4. 1.5 or 10ml disposable tubes.
5. 4°C storage area.

Methods

Concentrated protein assay

The linear range for this assay is from 0.25 mg/ml to 50 mg/ml protein per ml reagent. Therefore if your protein concentration exceeds this level in the final mixture then you will be underestimating the true concentration. The following formula is used as a general rule to estimate protein concentration:

1.0 OD_{600nm} = 100ug protein per ml reagent per cm light path length

For 95% of individually purified proteins this equation will estimate its concentration to within 20% of the true value. For protein extracts virtually all samples will be estimated to within 20% of the true value. For a more accurate determination, especially at low protein concentrations, it is recommended to use a standard curve of BSA protein.

Finally, for the odd cases, <5% of purified proteins, where the protein concentration is not the same as the true mass of protein added (one well known example is trypsin which reads approximately 10x lower than expected by mass), it is necessary to perform a standard curve of the actual protein, which can be used to then determine the true concentration of protein.

Method 1 for concentrated protein solutions (>3.0mg/ml)

1. Pipette 5ml of 1x Advanced Protein Assay Reagent into a 10ml disposable glass tube.
2. Pipette 5ul of protein solution into the tube containing 5ml and vortex 5 sec.
3. Blank the spectrophotometer on working solution and read absorbance of your sample at 600nm.
4. Calculate protein concentration based on 1.0 OD_{600nm} = 100ug protein per ml reagent per cm.

Method 2 for dilute protein solutions (<3.0mg/ml)

1. Pipette 1ml of 1x Advanced Protein Assay Reagent into a 1.5ml disposable microfuge tube.
2. Pipette 10ul of protein solution into the tube containing 1.0ml working solution.
3. Blank the spectrophotometer on working solution and read absorbance of your sample at 600nm.
4. Calculate protein concentration based on 1.0 OD_{600nm} = 100ug protein per ml reagent per cm.

Method 3 for concentrated protein solutions (>3mg/ml) in 96-well format:
1. Pipette 3.0ul of protein solution into two wells A1 and A2.
2. Pipette 0.3ml of 1x Advanced Protein Assay Reagent into wells A1, A2, B1 and B2.
3. Place in reader and measure absorbance at 600nm.
4. Calculate protein concentration based on 1.0 OD_{600nm} = 100ug protein per ml reagent per cm (for 96-well plates and 300ul wells, 300ul volume is equivalent to 0.8cm light path length).

Method 4 for dilute protein solutions (<3mg/ml) in 96-well format
1. Pipette 10ul of protein solution into two wells A1 and A2.
2. Pipette 0.3ml of 1x Advanced Protein Assay Reagent into wells A1, A2, B1 and B2.
3. Place in reader and measure absorbance at 600nm.
4. Calculate protein concentration based on 1.0 OD_{600nm} = 100ug protein per ml reagent per cm (for 96-well plates and 300ul wells, 300ul volume is equivalent to 0.8cm light path length).

6. Compatibility and interference levels

Chemical group / chemical name	Tested concentration that does Not alter protein assay color	Tested concentration that does not alter protein assay response to protein
Buffers / Tris pH 8.0	>1.0M	>1.0M
Buffers / Hepes pH 8.0	>1.0M	>1.0M
Buffers / Pipes pH 7.0	>1.0M	>1.0M
Buffers / Potassium phosphate pH7.0	>1.0M	>1.0M
Buffers / Sodium bicarbonate pH9.5	>1.0M	>1.0M
Reducing agents / BME	>1.0M	>1.0M
Reducing agents / DTT	>1.0M	>1.0M
Reducing agents / monothioglycerol	>1.0M	>1.0M
Denaturants / 8M urea	100%	100%
Denaturants / 5M guanidine-HCl	100%	100%
Divalent cations / MgCl2	>1.0M	>1.0M
Divalent cations / CaCl2	>1.0M	>1.0M
Divalent cations / NiCl2	>1.0M	>1.0M
Chelating agents / EDTA	>1.0M	>1.0M
Chelating agents / EGTA	>1.0M	>1.0M
Detergents / SDS	5.0%	0.5%
Detergents / NP40	0.5%	0.2%
Detergents / Triton X-100	0.2%	0.2%
Detergents / Tween 20	1.0%	0.5%
Solvents / DMSO	100%	100%
Solvents / DMF	100%	100%
Solvents / ethyl alcohol	100%	100%
Solvents / methanol	100%	100%
Antifoaming agent / Antifoam-C	30%	30%
Acids / hydrochloric acid	>1.0M	>1.0M
Acids / perchloric acid	>1.0M	>1.0M
Acids / trichloric acid	>1.0M	>1.0M
Acids / nitric acid	>1.0M	>1.0M
Acids / sulfuric acid	>1.0M	>1.0M

Trouble-shooting
1. Observation: No increase in blue color
Cause: Protein concentration too low or incorrect labeling of tubes.
Remedy: Use the method for dilute or very dilute protein solutions and Repeat assay.

2. Observation: During measurement of a large number of samples the standards read decreasing absorbance values
Cause: Time to read takes too long.
Remedy: Measure fewer samples at a time or use a high through put method for measurement (e.g. 96-well plates and eight channel pipettors).

3. Observation: Buffer blank reads too high absorbance values.
Cause: Buffer contains interfering chemicals (probably detergents).
Remedy: Use the concentrated protein assay or blank with interfering buffer or remove buffer prior to assay or use a detergentless buffer or ethanol precipitate proteins (3 volumes of ethanol), centrifuge (14000xg 10min) and resuspend in working solution.

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.