V. 4.0

Advanced Protein Assay Reagent

Cat. # ADV01

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Manual Contents

I: Introduction

Background

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 570 to 615 nm.

Uses

1) To determine protein concentration in a buffer.
2) To determine protein concentration in a biological fluid (e.g. serum or saliva).
3) To determine protein concentration in tissue culture media.
4) In combination with an activity assay this kit can be used to follow protein purification.

II: Purchaser Notification

Limited Use Statement

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of product and components of product in research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or any component thereof to a third party or otherwise use this product or its components for commercial purposes. Commercial purposes include, but are not limited to: use of the product or its components in manufacturing; use of the product or its components to provide a service; resale of the product or its components.

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III. Kit Contents

Contents:

1) 1 x 500 ml Advanced Protein Assay Reagent (5X concentrate; Cat # ADV01).

2) 1 x 50 ml tube for 1X dilution, store on bench at 24°C.

Equipment Required:

1) Spectrophotometer with 570 to 615 nm wavelength (optimally 600 nm).

2) Small volume capacity cuvettes (1.0 ml).

3) Pipettors 20 µl, 1000 µl and 5.0 ml capacity.

4) 1.5 or 10 ml disposable tubes.

5) 4°C storage area

Concentrated protein solutions (>3.0 mg/ml):

1. Pipette 5 ml of 1X Advanced Protein Assay Reagent (Cat. # ADV01) into a 10 ml disposable glass tube.
2. Pipette 5 µl of protein solution into the tube containing 5 ml ADV01 and vortex 5 s.
3. Blank the spectrophotometer on 1X ADV01 and read absorbance of your sample at between 570 and 615 nm (optimal 590 nm).
4. Calculate protein concentration based on 1.0 $OD_{570\ to\ 615\ nm}$ = 30 µg protein per ml reagent per cm.

Low concentration protein solutions (<3.0 mg/ml)

1. Pipette 1 ml of 1X Advanced Protein Assay Reagent (Cat. # ADV01) into a 1.5 ml disposable microfuge tube.
2. Pipette 10 µl of protein solution into the tube containing 1.0 ml ADV01.
3. Blank the spectrophotometer on 1X ADV01 and read absorbance of your sample at between 570 and 615 nm (optimal 590 nm).
4. Calculate protein concentration based on 1.0 $OD_{570\ to\ 615\ nm}$ = 30 µg protein per ml reagent per cm.

Note: For 96-well plates and 300 µl wells, a 300 µl volume is equivalent to 0.8 cm light pathlength.

Preliminaries:

1. Pipette 10 ml of 5X ADV01 into the 50 ml tube provided.
2. Pipette 40 ml of Milli-Q, ultrafiltered or distilled water into the same tube and mix.

This is your 1X stock of ADV01.

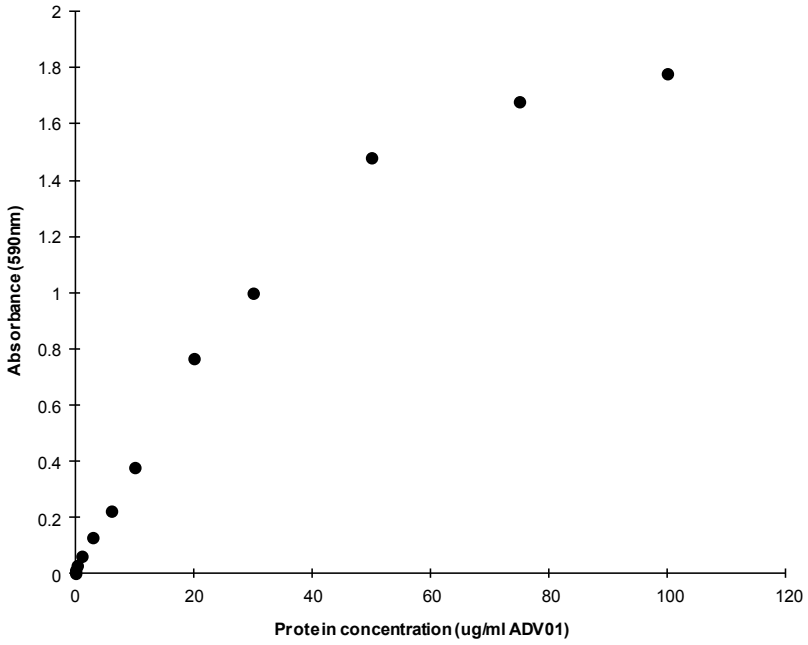
Concentrated protein assay:

The linear range for ADV01 is up to 40 µg 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 $OD_{570\ to\ 615\ nm}$ = 30 µg protein per ml reagent per cm light pathlength

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, as shown in Figure 1.

Figure 1: Standard curve of BSA protein using the Advanced Protein Assay Reagent



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.

Below there are five different methods for various applications, choose the one that most suits your application:

Method 1 for concentrated protein solutions (>3.0 mg/ml)
Method 2 for dilute protein solutions (<3.0 mg/ml)
Method 3 for very dilute protein solutions (<0.1 mg/ml)
Method 4 for concentrated protein solutions (>3 mg/ml) in 96-well format
Method 5 for dilute protein solutions (<3 mg/ml) in 96-well format

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

1. Pipette 5 ml of 1X Advanced Protein Assay Reagent (Cat. # ADV01) into a 10 ml disposable glass tube.
2. Pipette 5 µl of protein solution into the tube containing 5 ml ADV01 and vortex 5 s.
3. Blank the spectrophotometer on 1X ADV01 and read absorbance of your sample at between 570 and 615 nm (optimal 590 nm).
4. Calculate protein concentration based on 1.0 $OD_{570\ to\ 615\ nm}$ = 30 µg protein per ml reagent per cm.

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

1. Pipette 1 ml of 1X Advanced Protein Assay Reagent (Cat. # ADV01) into a 1.5 ml disposable microfuge tube.
2. Pipette 10 µl of protein solution into the tube containing 1.0 ml ADV01.
3. Blank the spectrophotometer on 1X ADV01 and read absorbance of your sample at between 570 and 615 nm (optimal 590 nm).
4. Calculate protein concentration based on 1.0 $OD_{570\ to\ 615\ nm}$ = 30 µg protein per ml reagent per cm.

Method 3 for very dilute protein solutions (<0.1 mg/ml):

1. Pipette 0.2 ml of 5X ADV01 into two 1.5 ml disposable microfuge tubes and label A and B.
2. Pipette 0.8 ml of protein solution into the tube A.
3. Pipette 0.8 ml of protein solution buffer only into the tube B.
4. Blank the spectrophotometer on contents of tube B, and read absorbance of your sample at between 570 and 615 nm (optimal 590 nm).
5. Calculate protein concentration based on 1.0 $OD_{570\ to\ 615\ nm}$ = 30 µg protein per ml reagent per cm.

Method 4 for concentrated protein solutions (>3 mg/ml) in 96-well format:

1. Pipette 3.0 µl of protein solution into two wells A1 and A2.

2. Pipette 0.3 ml of 1X ADV01 into wells A1, A2, B1 and B2.
3. Place in reader and measure absorbance at 570 to 615 nm, optimally 590 nm.
4. Calculate protein concentration based on 1.0 $OD_{570\ to\ 615\ nm}$ = 37.5 µg protein per ml reagent per 0.8 cm.

Method 5 for dilute protein solutions (<3 mg/ml) in 96-well format:

1. Pipette 10 µl of protein solution into two wells A1 and A2.
2. Pipette 0.3 ml of 1X ADV01 into wells A1, A2, B1 and B2.
3. Place in reader and measure absorbance at 570 to 615 nm, optimally 590 nm.
4. Calculate protein concentration based on 1.0 $OD_{570\ to\ 615\ nm}$ = 37.5 µg protein per ml reagent per 0.8 cm.

VI: Compatibility Table

Analysis of chemicals used in protein biochemistry that may interfere with ADV01 based assays.

Chemical group /chemical name	**Tested concentration that does not alter protein assay color (using 10 µl per ml ADV01)**	**Tested concentration that does not alter protein assay response to protein (using 10 µl per ml ADV01)**
Buffers / Tris pH 8.0	>1.0 M	>1.0 M
Buffers / HEPES pH 8.0	>1.0 M	>1.0 M
Buffers / PIPES pH 7.0	>1.0 M	>1.0 M
Buffers / Potassium phosphate pH7.0	>1.0 M	>1.0 M
Buffers / Sodium bicarbonate pH9.5	>1.0 M	>1.0 M
Reducing agents / BME	>1.0 M	>1.0 M
Reducing agents / DTT	>1.0 M	>1.0 M
Reducing agents / monothioglycerol	>1.0 M	>1.0 M
Denaturants / 8 M urea	100%	100%
Denaturants / 5 M guanidine-HCl	100%	100%
Divalent cations / $MgCl_2$	>1.0 M	>1.0 M
Divalent cations / $CaCl_2$	>1.0 M	>1.0 M
Divalent cations / $NiCl_2$	>0.1 M	>0.1 M
Chelating agents / EDTA	>1.0 M	>1.0 M
Chelating agents / EGTA	>1.0 M	>1.0 M
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	>1.0 M	>1.0 M
Acids / perchloric acid	>1.0 M	>1.0 M
Acids / trichloric acid	>1.0 M	>1.0 M
Acids / nitric acid	>1.0 M	>1.0 M
Acids / sulfuric acid	>1.0 M	>1.0 M

VII: Troubleshooting

Observation	Possible cause	Solution
1. No increase in blue color	1. Protein concentration too low 2. Incorrect labeling of tubes	1. Use the method for dilute or very dilute protein solutions. 2. Repeat assay.
2. During measurement of a large number of samples the standards read decreasing absorbance values.	1. Time to read takes too long.	1. Measure fewer samples at a time. 2. Or use a high through put method for measurement (e.g. 96-well plates and eight channel pipettors).
3. Buffer blank reads too high absorbance values.	1. Buffer contains interfering chemicals (probably detergents).	1. Use the concentrated protein assay. 2. Use a detergent free buffer. 3. Or ethanol precipitate proteins (3 volumes of ethanol), centrifuge (14000 x g 10 min) and resuspend in 1X ADV01.

VIII: Notes on Updated Version

Notes on Updated Version 4.0

A. Manual updated to new manual format.

NOTES:

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