V. 7.0

Precision Red Advanced Protein Assay Reagent

Cat. # ADV02

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Manual Contents

I: Introduction

Background

The Precision Red Advanced Protein Assay Reagent (Cat# ADV02) is designed to extend the linear range of protein measurement, a limiting factor in other protein assays. The reagent combines the useful properties of low protein to protein variance and a broad linear response to many types of purified protein. A simple one step procedure results in a red to purple/blue color change characterized by an increase in absorbance at 580 to 620 nm.

Uses

1) To determine protein concentration in a variety of common buffers.
2) To determine protein concentration in biological fluids (e.g. serum or saliva).
3) To determine protein concentration in tissue culture media.
4) To follow protein purification steps in combination with activity assays.

II: Purchaser Notification

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III. Kit Contents

Contents: 1 x 500 ml Advanced Protein Assay Reagent (1X concentrate; Cat # ADV02). Store at room temperature for up to 6 months.

Equipment Required:

1) Spectrophotometer capable of measuring 580 to 620 nm wavelength (optimally 600 nm)

2) Small volume cuvettes (1.0 ml) or 96-well plates.

3) Pipettors 3-20 µl, 100-1000 µl and 10 ml capacity.

4) 1.5 or 15 ml disposable tubes.

A) Protein solutions between 0.3 to 10 mg/ml

1. Pipette 1 ml of ADV02 into a 1.5 ml disposable microfuge tube.
2. Pipette 10 µl of protein solution into the tube containing 1.0 ml ADV02 and mix by inverting.
3. Incubate at room temperature for one minute.
4. Blank the spectrophotometer on ADV02 at 600 nm and read absorbance of your sample.
5. Calculate protein concentration based on 1.00 OD600 nm = 100 µg protein per ml reagent per cm, and multiply this protein concentration by 100 to achieve the protein concentration in µg/ml of original protein solution.

B) Low concentration protein solutions (<0.3 mg/ml)

1. Pipette 900 µl of ADV02 into a 1.5 ml disposable microfuge tube.
2. Pipette 100 µl of protein solution into the tube containing 900 µl ADV02 and mix by inverting.
3. Incubate at room temperature for one minute.
4. Blank the spectrophotometer on 1 ml of 900 µl ADV02 plus 100 µl of your buffer at 600 nm and read absorbance of your sample.
5. Calculate protein concentration based on 1.00 OD600 nm = 100 µg protein per ml reagent per cm, and multiply this protein concentration by 10 to achieve the protein concentration in µg/ml of original protein solution.

C) High concentration protein solutions (10-100 mg/ml):

1. Pipette 10 ml of ADV02 into a 15 ml disposable plastic tube.
2. Pipette 10 µl of protein solution into the tube containing 10 ml ADV02 and mix thoroughly.
3. Incubate at room temperature for one minute.
4. Blank the spectrophotometer on ADV02 and read absorbance of your sample at 600 nm.
5. Calculate protein concentration based on 1.00 OD600 nm = 100 µg protein per ml reagent per cm, and multiply this protein concentration by 1000 to achieve the protein concentration in µg/ml of original protein solution.

Note: For 96-well plates and 300 µl wells, a 300 µl volume is equivalent to 0.8 cm light pathlength. See below for the method suited to 96-well plate technology.

VI: Detailed Methods

A) Description of the assay characteristics

The linear range of the ADV02 assay is between 0.05 OD600 nm to 0.80 OD600 nm, either side of this range will result in inaccurate readings. The standard factor (1.00 OD600 nm is equivalent to 100 µg protein per ml of reagent) is used to calculate protein concentration based on the average value obtained from several different proteins. The highest value is found with BSA where an OD of 1.00 is equivalent to 90 µg/ml, and the lowest with IgG where an OD of 1.00 is equivalent to 130 µg/ml. Greater than 95% of tested proteins have been found to be located between these values.

B) Standard Assay

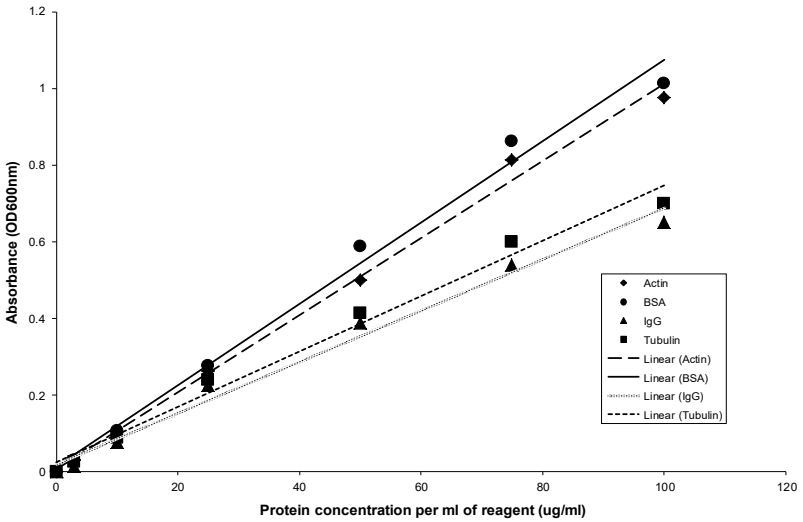
The linear range for ADV02 is up to 100 µg protein per ml reagent. Therefore if your protein concentration exceeds this level in the final mixture then you will be underestimating its true concentration. The following formula is used as a general rule to estimate protein concentration:

1.00 OD 600 nm = 100 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 IgG protein, as shown in Figure 1.

Figure 1: Standard curves of Actin, BSA, IgG and Tubulin protein using the Precision Red Advanced Protein Assay Reagent



C) Method for measurements in 96-well format:

1. Pipette either 3 or 10 µl of protein solution into two wells A1 and A2.
2. Pipette 0.3 ml of ADV02 into wells A1, A2, B1 and B2.
3. Incubate at room temperature for one minute.
4. Place in reader and measure absorbance at 600 nm (using 580 and 620 nm is possible but the sensitivity will decrease approximately two fold when approaching these limits).
5. Calculate protein concentration based on 1.00 OD600 nm = 125 µg protein per ml reagent, and multiply this concentration by 100 (for 3 µl) or 30 (for 10 µl) to achieve ug protein per ml of original sample. Remember to not use ODs above 1.00 which will be off the standard curve.

VI: Compatibility Table

Analysis of chemicals used in protein biochemistry that may interfere with ADV02 based assays.

Chemical group /chemical name	Tested concentration that does not alter protein assay color (using 10µl per ml ADV02)	Tested concentration that does not alter protein assay response to protein (using 10µl per ml ADV02)
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	0.1%	0.1%
Detergents / NP40	10%	10%
Detergents / Triton X-100	10%	0.5%
Detergents / Tween 20	10%	10%
Solvents / DMSO	100%	100%
Solvents / DMF	100%	100%
Solvents / ethyl alcohol	100%	100%
Solvents / methanol	100%	100%
Antifoaming agent / Antifoam-C	10%	10%
Acids / hydrochloric acid	>1.0 M	>0.75 M
Acids / perchloric acid	>1.0 M	>0.75 M
Acids / trichloric acid	>1.0 M	>0.50 M
Acids / nitric acid	>1.0 M	>0.75 M
Acids / sulfuric acid	>1.0 M	>0.25 M

VII: Troubleshooting

Observation	Possible cause	Solution
1. No increase in blue color	1. Protein concentration too low	1. Use the method for dilute or very dilute protein solutions. For protein solutions of less than 0.5 mg/ml it is recommended to use ADV01 which is excellent for detecting low protein concentrations and a good linear response in these low protein concentrations ranges.
	2. Incorrect labeling of tubes	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, i.e. greater than 30 min.	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 (>0.05 OD) absorbance values when compared to ADV02 alone.	1. Buffer contains interfering chemicals (probably detergents).	1. Use the concentrated protein assay. 2. Use a detergent free buffer. 3. Ethanol precipitate proteins (3 volumes of ethanol), centrifuge (14000 x g 10 min) and resuspend in ADV02.

VIII: Notes on Updated Version

Notes on Updated Version 7.0

Minor grammatical corrections.

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