Kushan Zist

Bradford Protein Assay Kit

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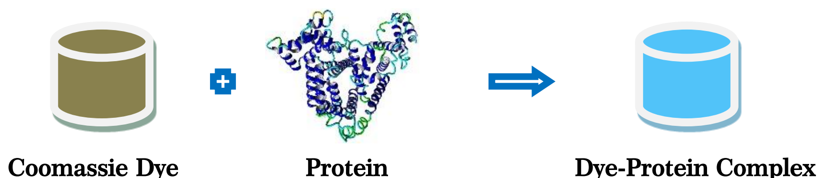
About This Kit

The Bradford assay for protein quantitation has become the preferred method for quantifying protein in many laboratories as the technique is simple, rapid, sensitive and relatively free of interference from the most common chemical reagents and nonprotein components of biological samples.
This product is a microplate-based version of the Bradford's famous assay, which utilizes the Coomassie Blue G, that binds to the amino acids (mainly basic and aromatic) of proteins in an acidic medium, forming a blue complex. The resulted blue dye-protein complex is proportional to the amount of protein in the sample and can be quantified photometrically (595 nm).



Kit Components

Item Label	Amount
BSA Standard	1.5 ml
Dye Reagent	10 ml
96 Well Microplate	2 Plate
Instruction Manual	1 Manual

Storage and Stability

- This kit will perform as specified if **stored at 4°C**.
- Use before the **expiration date** indicated on the box.

Kit Performance

- **Standard assay range:** 0.125-1.0 mg/ml
- **Micro assay range:** 5-30 μg/ml
- **Sensitivity:** 1 μg/ml
- **Application:** For *in vitro* measurement of total protein in aqueous solutions.

Materials Required (Not Provided)

- Double distilled water (ddH_2O)
- Microtubes, 15 ml / 50 ml tubes

Required Instrumentation

- Multiwell microplate reader capable of measuring absorbance at 595 ± 15 nm
- Microfuge
- Pipettes with variable volume setting.
- Plate shaker or Orbital shaker

Warning and Precautions

- It is recommended that gloves, lab coat, and protective eyewear be worn at all times.
- Consider all components as hazardous and dispose according to established safety laws.

- The Dye Reagent contains concentrated corrosive and toxic chemicals. In case of contact with eyes or skin, rinse with plenty of water for several minutes and get medical attention.

Reagent Preparation

Assay Reagent: Invert the Dye Reagent bottle a few times. Prepare the Assay Reagent by diluting 7.5 ml of the Dye Reagent with 42.5 ml of ddH_2O in a fresh tube (final volume is 50 ml). The Assay Reagent is stable for up to one year if stored in the dark at 4°C.

Note: Allow the Assay Reagent to equilibrate to room temperature and invert several times immediately before use in your formal assay.

STANDARD PREPRATION

Note: In any protein assay, the best protein to be used as a standard is the purified form of the protein being assayed.

Note: The diluted standards should not be used after 24 hours.

For Standard Assay

The BSA Standard is provided at a stock concertation of 5 mg/ml. Dilute 280 µl of the stock standard with 420 µl of ddH_2O to prepare 700 µl of a bulk standard of 2 mg/ml in concentration.

Take seven fresh microtubes and label them A-G. Add the amount of bulk standard (2 mg/ml) and ddH_2O to each tube as described below.

Tubes	2 mg/ml Bulk Standard (µl)	ddH_2O (µl)	Final Concentration (µg/ml)
A	150	250	750
B	125	275	625
C	100	300	500
D	75	325	375
E	50	350	250
F	25	375	125
G	0	400	0

For Micro-Assay

The BSA Standard is provided at a stock concertation of 5 mg/ml. Dilute 40 µl of the stock standard with 960 µl of ddH_2O to prepare 1 ml of a bulk standard of 0.2 mg/ml in concentration.

Take seven fresh microtubes and label them A-G. Add the amount of bulk standard (0.2 mg/ml) and ddH_2O to each tube as described below.

Tubes	0.2 mg/ml Bulk Standard (µl)	ddH_2O (µl)	Final Concentration (µg/ml)
A	60	340	30
B	50	350	25
C	40	360	20
D	30	370	15
E	20	380	10
F	10	390	5
G	0	400	0

Things to Note

- Allow all reagents to equilibrate to room temperature prior to assay.
- Assay all samples and standards in duplicate/triplicate.
- Pay close attention to your pipetting technique to avoid erratic results in sample replicates.
- A standard curve must be run simultaneously with each set of samples.
- If the protein content of the samples is beyond the range of the standard curve, the samples should be assayed at several dilutions.
- If a particular reagent interferes with an assay, prepare a standard curve with protein diluted with the reagent in question. Run a side-by-side comparison of this standard curve with one that has been prepared with ddH_2O.
- Plastic and glassware used in the assay should be absolutely clean and detergent free.
- Traces of dye bound to glassware or plastic can be removed by rinsing with methanol or detergent solution.

Assay Protocol

Standard assay protocol

Note: The linear range of BSA in the standard assay format is 125–1,000 µg/ml.

1. Shake all samples to homogenize.
2. Add 10 µl of standards or samples to related wells.
3. Add 190 µl of Assay Reagent to all wells (*multichannel pipettes are preferred at this step to reduce dispensing time*).
4. Shake gently and incubate for 5 minutes at room temperature.
5. Using a plate reader, read the absorbance at 595 ± 15 nm within one hour.

Micro-assay protocol

Note: The linear range of BSA in the micro-assay format is 5–30 µg/ml.

1. Shake all samples to homogenize.
2. Add 100 µl of standards or samples to related wells.
3. Add 100 µl of Assay Reagent to all wells (*multichannel pipettes are preferred at this step to reduce dispensing time*).
4. Shake gently and incubate for 5 minutes at room temperature.
5. Using a plate reader, read the absorbance at 595 ± 15 nm within one hour.

Note: The color of the dye is expected to remain stable for at least one hour, but dye-dye aggregates may form during this period, so take readings as soon as possible.

Calculations

- Calculate the average absorbance of each standard and sample.
- Subtract the absorbance value of the standard G (0 mg/ml) from itself and all other values (both standards and samples). This is the corrected absorbance.
- Plot the corrected absorbance values of each standard as a function of BSA concentration.
- Calculate the values of total protein for each sample from the standard curve.

$$\text{Total protein } (\mu\text{g/ml}) = \frac{(Corrected\ absorbance) - (Intercept)}{Slope} * sample\ dilution$$

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low absorbance in samples and standards	Reagent cold or stored improperly	Allow reagent to warm to room temp.
	Absorbance not measured at 595nm	Absorbance may be read at any wavelength between 575 nm to 615 nm
Precipitate form in all wells	Sample contains a detergent	Dialyze or dilute sample to remove or decrease detergent
	Samples not mixed well or allowed to stand for several hours before reading	Mix samples by pipetting several times prior to reading
Dark blue appears in all sample wells	Concentrated samples	Dilute samples and re-assay

Compatible Reagents

Reagent	Concentration	Reagent	Concentration
2-Mercaptoethanol	1 M	NaCl	2.5 M
Acetonitrile	10%	NaN_3	0.5%
Ascorbic acid	50 mM	NaOH	0.1 M
$CaCl_2$	40 mM	Na_3PO_4	0.5 M
DMSO	5%	$(NH_4)_2SO_4$	1 M
EDTA	0.2 M	PBS	
Ethanol	10%	PMSF	2 mM
Glucose	20%	SDS	0.025%
Glycerol	5%	Sodium acetate, pH 4.8	0.2 M
Hank's salt solution		Sodium carbonate	0.1 M
HCl	0.1 M	Sodium citrate, pH 4.8 or 6.4	0.2 M
HEPES	0.1 M	Sucrose	10%
KCl	2 M	Tris	1 M
K_3PO_4	0.5 M	Triton X-100	0.05%
$MgCl_2$	1 M	Tween 20	0.01%
Methanol	10%	Urea	4 M

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