Technical Service Contact Information
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Hours: S-T 8:00 AM to 3:30 PM

TEB PAZHOUHAN RAZI

(TPR)

Bradford Assay Kit

(200 Tests)

Storage and Stability

This kit will perform as specified if **stored at 4°C**
Use before the **expiration date**

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About This Kit

TPR's Bradford assay Kit is a simple and rapid tool for determining total protein in aqueous medium. It takes advantage of the color change of Coomassie dye when binds to proteins in acidic medium. The resulted blue dye-protein formation can simply be quantified colorimetrically (595 nm).

Components

Item Label	Item	Quantity
Reagent 1 **(R1)**	Contains BSA Standard	5mg
Reagent 2 (**R2**)	Contains 5X TPB Reagent	8 ml
-	96 Well Microplate	1 Plate
-	Technical Manual	1 Manual

Performance

- **Linear Range:** 100-800 µg/ml.

Required Materials Not Provided

- Distilled water or HPLC-grade water.
- 1.5 ml microtubes.
- 15 ml/50 ml fresh tube.

Required Instrumentation

- Microplate reader capable of measuring absorbance at 595 nm.
- Adjustable pipettes.
- Plate shaker/Orbital shaker

Warning and precautions

- Reagent 2 (R2) contains corrosive and toxic chemicals. In case of contact with eyes or skin, rinse immediately with large amounts of water for few minutes and get medical attention.
- Always wear suitable protective clothing.

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Reagent Preparation

- **Ready R1 Reagent: Spin R1** vial for few secounds and reconstitute with 1000 µl of distilled water to obtain a stock solution of 5 mg/ml. Ready R1 reagent should be aliquoted into 4 vials after reconstitution (0.25 ml in each vial) and stored at -20°C for long term storage.

- **1X Ready R2 Reagent: Shake R2** bottle for few secounds. Prepare 1X Ready R2 Reagent by diluting the 5X **R2** with distilled water in a fresh tube (Mix 1 part R2 with 4 part distilled water). This reagent is stable for 24 hours and Left-overs should be discarded, hence only prepare enough 1X Ready R2 Reagent for a one-day experiment.

Standard Preparation

- Take seven clean 0.5 ml microtubes and label them A-G. Add the amount of Ready R1 Reagent and distilled water to each tube as described below. These diluted standards should not be used after 24 hours.

Tube	R1 Reagent (µl)	Water (µl)	BSA Concentration (µg/ml)
A	80	420	800
B	60	440	600
C	40	460	400
D	30	470	300
E	20	480	200
F	10	490	100
G	0	500	0

Things to Note

- Allow all reagents to equilibrate to room temperature before performing the assay.
- It is recommended that all samples and standards be assayed at least in duplicate (triplicate recommended).
- A standard curve must be run simultaneously with each set of samples.
- If the protein content of samples are beyond the range of standard curve, samples must be assayed at several dilutions. NOTE: *It has been our experience that samples resulting from the homogenization of tissues need to be diluted by at least a factor of 100 (i.e., 10 µl of sample + 990 µl of water).*

Assay Protocol

1. Shake all samples for homogenation.
2. Add 10 µl standards/samples to each wells.
3. Add 190 µl of 1X Ready R2 Reagent to all wells (multichannel pipettes are preferred at this step to reduce dispensing time).
4. Shake gently to mix properly and incubate for 5 minutes at room temperature.
5. Measure absorbance at 595 nm immediately.

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Note: The dye color expected to be stable for at least one hour but dye-dye aggregates may form during this period, hence perform the readings immediately.

Calculations

1. Calculate the average absorbance of each standard and sample.
2. 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.
3. Plot the corrected absorbance values of each standard as a function of BSA concentration.
4. Calculate the values of total protein for each sample from the standard curve.

$$\boldsymbol{Total\ Protein}\ (\boldsymbol{mg/ml}) = \frac{(Corrected\ Absorbance) - (y - intercept)}{Slope} * sample\ dilution$$

Troubleshooting

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

References

- Bradford, M.M. Anal Biochem. (1976) 72:248-54.
- Compton, S.J. and Jones, C.J. Anal Biochem. (1985)151:369-74.
- Tal, M., Silberstein, A. and Nusser, E. Anal Biochem. (1980) 79:544-52.