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BIO BASIC

Bradford Protein Assay Kit

SK3031
Version 2017AM1
Rev 12.10.2017

For Research Use Only

Bradford Protein Assay Kit

Code: SK3031

Components	Quantity
Bradford Reagent	200 ml
PBS	10 ml
BSA Standard Solution (5 mg/ml)	1 ml
Protocol	1 pc

Description

Bradford method utilizes Coomassie Brillant Blue G-250 dye binding to an unknown protein and forming a complex which can be detected spectophotometrically at 595 nm. This kit is designed to quantitate 10-150 µg/ml of protein.

Features

√ Rapid and economical: entire procedure (10-20 samples) only takes about 10 minutes (time for preparation of samples is not included).
√ Good linear relationship between 10 µg/ml to 150 µg/ml protein range.
√ High sensitivity: the minimum protein quantity which could be tested is 0.2 µg in 1-20 µl volume.

Application

Direct assays of total protein concentration.

Other Kits Available:

1. **SK3041**: Better Bradford Protein Assay kit (1000 Assays)
2. **SK3021**: BCA Protein Assay Kit (Smith Assays) (500 Assays)
3. **SK3051**: Better BCA Protein Assay Kit (500 Assays)
4. **SK4031**: Lowry Protein Assay Kit (1000 Assays)

Notes:

1. Mix the Bradford Reagent thoroughly before use.
2. Warm the Bradford Reagent to RT before use.
3. If there is detergent present in the sample, please use BCA Protein Assay Kit (SK3021, SK3051).
4. Absorbance of protein varies; the results will be more accurate if the protein of interest were used to obtain the standard curve.
5. Measure samples in duplicate to reduce error, plot standard curve every time.
6. Measure samples with the lowest concentration first.

Protocols for Measurements in Test Tubes

A. Make Dilutions of BSA standards and Sample of interest.

Label 8 test tubes from #1 to #8. Make BSA serial dilutions as follows:

In Tube #1, add 20 µl of original BSA protein standard (5 mg/ml) + 780 µl of PBS.
In Tube #2, transfer 100 µl of PBS + 400 µl from Tube #1.
In Tube #3, transfer 100 µl of PBS + 400 µl from Tube #2.
In Tube #4, transfer 120 µl of PBS + 360 µl from Tube #3.
In Tube #5, transfer 120 µl of PBS + 240 µl from Tube #4.
In Tube #6, transfer 150 µl of PBS + 150 µl from Tube #5.
In Tube #7, transfer 100 µl of PBS + 100 µl from Tube #6.
In Tube #8, transfer 100 µl of PBS ONLY.

This is a BSA standard serial dilution

no.	1	2	3	4	5	6	7	8
BSA final concentration (ug/ml)	125	100	80	60	40	20	10	0

Now dilute sample of interest to preferred concentration (10-125 µg/ml). Record dilution factor X.

Label Tubes #9 to #11

In Tube #9, transfer 100 µl of diluted protein sample of interest.
In Tube #10, transfer 100 µl of diluted protein sample of interest, preferred at a different dilution factor.
In Tube #11, transfer 100 µl of PBS ONLY.

B. Measure concentration of BSA standards and sample of interest

1. Transfer **100 µl** of **BSA serial dilutions** (step A) from each tube #1 to #8 to a new clean tube. Make duplicates as a good laboratory practice. Add **1 ml** of Bradford Reagent to each tube. Mix well and incubate samples for 10 minutes at room temperature (RT).
2. With the spectrophotometer set to 595 nm, zero the instrument on a cuvette filled only with PBS. Subsequently, measure the absorbance of all the samples within 10 minutes.
3. Subtract the average 595 nm absorbance measurement of the Blank standard replicates from the 595 nm absorbance measurement of all other individual standard and unknown sample replicates.
4. Plot standard curve using the average Blank-corrected 595 nm measurement for each BSA standard vs. its concentration in µg/ml.
5. Using BSA protein standard curve, calculate concentration of diluted protein sample and original protein concentration with dilution factor X.

Protocols for Measurements In 96-well Plate

A. Make Dilutions of BSA standards and Sample of interest.

Label 8 test tubes from #1 to #8. Make BSA serial dilutions as follows:

In Tube #1, add 3 µl of original BSA protein standard (5 mg/ml) + 117 µl of PBS.
In Tube #2, transfer 20 µl of PBS + 80 µl from Tube #1.
In Tube #3, transfer 15 µl of PBS + 60 µl from Tube #2.
In Tube #4, transfer 15 µl of PBS + 45 µl from Tube #3.
In Tube #5, transfer 15 µl of PBS + 30 µl from Tube #4.
In Tube #6, transfer 15 µl of PBS + 15 µl from Tube #5.
In Tube #7, transfer 10 µl of PBS + 10 µl from Tube #6.
In Tube #8, transfer 20 µl of PBS ONLY.

This is a BSA standard serial dilution

no.	1	2	3	4	5	6	7	8
BSA final concentration (ug/ml)	125	100	80	60	40	20	10	0

Now dilute sample of interest to preferred concentration (10-125 µg/ml). Record dilution factor X.

Label Tubes #9 to #11

In Test Tube #9, transfer 20 µl of diluted protein sample of interest.
In Tube #10, transfer 20 µl of diluted protein sample of interest, preferred at a different dilution factor.
In Tube #11, transfer 20 µl of PBS ONLY.

B. Measure concentration of BSA standards and sample of interest

1. Transfer **20 µl** of BSA serial dilutions (step A) from each tube #1 to #8 to each well in a 96-well plate. Make duplicates as a good laboratory practice. Add **200 µl** of Bradford Reagent to each tube. Mix with the plate shaker for 30 seconds and incubate plate for 10 minutes at room temperature (RT).
2. Measure the absorbance at 595 nm on a plate reader.
3. Subtract the average 595 nm absorbance measurement of the Blank standard replicates from the 595 nm absorbance measurement of all other individual standard and unknown sample replicates.
4. Plot standard curve using the average Blank-corrected 595 nm measurement for each BSA standard vs. its concentration in µg/ml.
5. Using BSA protein standard curve, calculate concentration of diluted protein sample and original protein concentration with dilution factor X.