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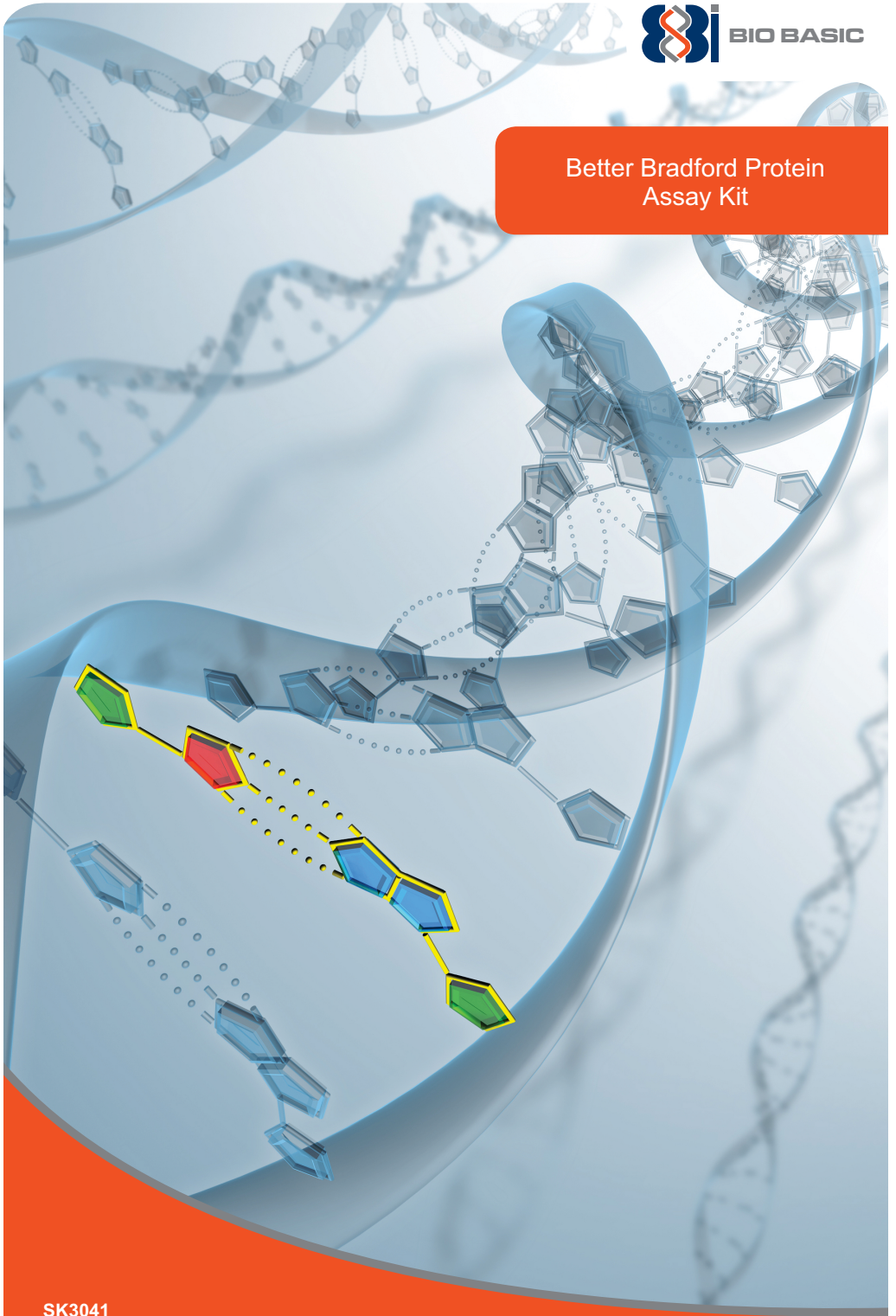
Better Bradford Protein Assay Kit



SK3041
QF 24 TV4
CV1 2020

For Research Use Only

Better Bradford Protein Assay Kit

Code: SK3041 (1000 Preps)

Description

The Better Bradford Protein Assay Kit is a quick and ready-to-use coomassie-binding, colorimetric method for total protein quantitation. This modification of the well-known Bradford method greatly reduces the tendency of coomassie reagents to give nonlinear response curves by a formulation that substantially improves linearity for a defined range of protein concentration. In addition, the kit results in significantly less protein-to-protein variation than is observed with other Bradford-type coomassie formulations. When coomassie dye binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465 nm to 595 nm with a concomitant color change from brown to blue. Performing the assay in either test tube or microplate format is simple: combine a small amount of protein sample with the assay reagent, mix well, incubate briefly and measure the absorbance at 595 nm. Protein concentrations are estimated by reference to absorbance obtained from a series of standard protein dilutions, which are assayed alongside the unknown samples.

Features

The kit contains additives that retard the formation of dye-dye and dye-protein aggregates, so reduce the tendency of the reagent to nonlinear response curves by improving the formulation to be more sensitive and stable.

5. When samples contain detergent, dialyze or dilute the sample before carrying out protein quantitation.

2. Transfer 150 µl of diluted protein sample solution and 150 µl of the BSA Standards from above mixed solution (step A.1) in tube number 0 and tube number 1-6 into each well.
3. Add 150 µl of Bradford reagents into each well, then vortex to mix.
4. Keep them at room temperature for 10 minutes, invert 1-2 times.
5. Take the mixed solution of tube number 0 as reference, measure the A_{595} absorbance of each tube using a spectrophotometer.
6. Repeat the steps 2-5 once more, calculate the average A_{595} absorbance of tubes with same numbers.
7. Plot the BSA protein standard curve (A_{595} absorbance of tube 1-6 on the y-axis).
8. Calculate the average A_{595} absorbance of the diluted protein sample, and figure out the concentration of the diluted sample using the BSA protein standard curve.

Notes

1. Remove the Bradford reagent from 4°C storage and let it warm to ambient temperature. Invert the reagent a few times before use.
2. The concentration of samples must within the range of BSA standard curve, otherwise the samples need to be diluted or concentrated.
3. After adding Bradford reagent, measure the A_{595} absorbance within 30 minutes.
4. Samples that contain hydrophobic group and stick protein can form precipitates, add the same volume 1M NaOH to sample solution to dissolve the precipitate.

Kit Contents

The kit is composed of Bradford reagent and BSA standard protein, spectrophotometer 200 assays or microplate 1000 assays.

Components	
Bradford Reagent	200 ml
BSA standard protein (1 mg/ml)	5 ml

Storage

Keep the Bradford reagent at 2-8ºC and BSA protein at -20ºC.

Procedures

A) Plot the BSA Serial Dilution

1. Take twelve 1.5 ml centrifuge tubes; transfer the corresponding volume solution listed in the following chart into those 1.5 ml centrifuge tubes.

Solution Added \ Tube Number	0	1	2	3	4	5	6	7	8	9	10	11
ddH2O (µl)	1000	995	990	985	980	975	970	900	850	800	750	700
BSA standard (µl)	0	5	10	15	20	25	30	100	150	200	250	300
BSA final concentration (µg/ml)	0	5	10	15	20	25	30	100	150	200	250	300

B) Standard Test Tube Protocol (Working Range = 100-300 µg/ml)

1. Dilute the protein sample into suitable volume.
2. Take several 1.5 ml centrifuge tubes; add 100 µl of diluted protein sample solution, and 100 µl of the BSA Standards from above mixed solution (step A.1) in tube number 0 and tube number 7-10.

3. Add 1 ml Bradford reagents into each centrifuge tube, then vortex.
4. Keep them at room temperature for 10 minutes, invert 1-2 times.
5. After 10 minutes, take the mixed solution of tube number 0 as reference, measure the A_{595} absorbance of each tube using a spectrophotometer.
6. Repeat steps 2-5 once more, calculate the average A_{595} absorbance of tubes with same number.
7. Plot the BSA protein standard curve (A_{595} absorbance of tube 7-11 on the y-axis).
8. Calculate the average A_{595} absorbance of the diluted protein sample, and figure out the concentration of the diluted sample using the BSA protein standard curve.

C) Standard Test Tube Protocol (Working Range = 5-30 µg/ml)

1. Dilute the protein sample into suitable volume.
2. Take eight 1.5 ml centrifuge tubes, add 500 µl of diluted protein sample solution, and 500 µl of the BSA Standards from above mixed solution (step A.1) in tube number 0 and the tube number 1-6.
3. Add 0.5 ml Bradford reagent into each centrifuge tube, then vortex to mix.
4. Keep them at room temperature for 10 minutes, invert 1-2 times.
5. After 10 minutes, take the mixed solution of tube number 0 as reference, measure the A_{595} absorbance of diluted protein sample and the tube number 1-6 number using a spectrophotometer.
6. Repeat steps 2-5 once more, calculate the average A_{595} absorbance of tubes with same number.

7. Plot the BSA protein standard curve (A_{595} absorbance of tube 1-6 on the y-axis).
8. Calculate the average A_{595} absorbance of the diluted protein sample, and figure out the concentration of the diluted sample using the BSA protein standard curve.

D) Microplate Protocol (Working Range = 100-300 µg/ml)

1. Dilute the protein sample into suitable volume.
2. Transfer 20 µl of the diluted protein sample solution, and 20 µl of the BSA Standards from above mixed solution (step A.1) in tube number 0 and tube number 7-11 into each well.
3. Add 0.2 ml of Bradford reagent into each well, then vortex to mix.
4. Keep them at room temperature for 10 minutes, invert 1-2 times.
5. After 10 minutes, take the mixed solution of tube number 0 as reference, measure the A_{595} absorbance of each tube using a spectrophotometer.
6. Repeat the step 2-5 once more, calculate the average A_{595} absorbance of tubes with same numbers.
7. Plot the BSA protein standard curve (A_{595} absorbance of tube 7-11 on the y-axis).
8. Calculate the average A_{595} absorbance of the diluted protein sample, and figure out the concentration of the diluted sample using the BSA protein standard curve.

E) Microplate Protocol (Working Range = 5-30 µg/ml)

1. Dilute the protein sample into suitable volume.