DNAbiotech

Biotechnology is our expertise

Bradford Protein Assay Kit

For the rapid, sensitive and accurate measurement of protein in various samples.

Catalog no.: DB0017

Unit Size: 625, 1250 and 2500 assay

Related products:

5X Bradford Reagents: DB9701

BCA Kit: DB9684

Intended for research use only

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Customer and technical support

If you have any question, do not hesitate to ask! DNAbiotech would be highly appreciated for any comment(s).

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Quality Control

In accordance with DNAbiotech Co. Management System, all of the products are tested against predetermined specifications to ensure consistent product quality.

Safety Notes

The Bradford assay reagent (5X) in DNAbiotech **Bradford protein assay kit** contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

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General description

The Brad Ford protein assay method provides a simple and rapid procedure for determining the concentration of protein in solution. The method utilizes an improved Coomassie dye reagent which forms a blue complex in the presence of protein. The principle of this assay is that the binding of protein molecules to Coomassie dye under acidic conditions results in a color change from brown to blue. This method actually measures the presence of the basic amino acid residues, arginine, lysine and histidine, which contributes to formation of the protein-dye complex. The intensity of the blue complex is proportional to the amount of protein in the sample and can be easily measured by spectrophotometer or plate reader at 595 nm. Unlike the BCA assay, reducing agents (*i.e.,* DTT and beta—mercaptoethanol) and metal chelators (*i.e.,* EDTA, EGTA) at low concentration do not cause interference. However, the presence of **SDS (sodium dodecyl sulfate)** even at low concentrations can interfere with protein-dye binding. This technique was invented by Bradford.

Kit Components: (Table 1.)

No.	Item	Quantity
1	Bradford assay reagent (5X)	30 ml, 58ml and 114 ml
2	BSA powder (10 mg)	1 vial plus 10 vials and labels
3	Ready to Use PBS powder for 500 ml	1 pack
4	96 well ELISA plate	1 pcs.
5	Numbered vials for BSA standard preparation	7 pcs.
6	handbook	Pleas download form site.

Storage upon receipt:

- 2-8 °C
- Protect from light

Note 1: after reconstitution of BSA, standards vials should be kept in -20° C.

Note 2: Delivery can be done at room temperatures.

Procedure of the Bradford assay

1. Precautions before use

The following are precautions for the use of this product. Please read the handbook completely before use:

- Dissolve the ready to use powder of PBS (cat#: DB0010) in 500 mL of DDW. 1X PBS buffer can be store at 4° C for 6 months.
- Add 1 mL of PBS into the BSA vial to reconstitute BSA **(concentration: 10 mg/mL).** You can transfer 100 ul of the reconstitute BSA in 1 ml microtubes and add 900 ul of PBS (or 0.9% NaCl) and keep them **at -20° C.** By this you will have 10 stock vials of **1 mg/mL** BSA **(Final concentration: 1 mg/mL).**
- If a 595 nm filter is not available, measurement can be performed using a 575-620 nm filter. Doing so will have no effect on the results of quantification.
- Glass or quartz cuvettes must be thorough cleaned with ethanol or methanol after use as coomassie dye can stain. Disposable polystyrene cuvettes are a convenient alternative.

2. Standard Curve Preparation

According to the concentration of the sample chose one of the following tables.

Note 1: Please bring the Bradford assay reagent (5X) to room temperature before use and mix it gently by inverting the bottle 5 times. (Do not shake the bottle to mix the solution). **Working solution of this reagent is 1X**, so according to your

need, it **should be diluted by DW before** use (e.g. if you need 5 ml, add 4 ml DW to 1 ml of 5X reagent and mix).

Note 2: The assay is performed as duplicated or triplicate determination.

Note 3: According to the results, the calibration curve should be created new time to time.

Note 4: If needed, the final volume of the standard vials could be double.

2-1: Dilute the BSA Standard to 1 mg/ml stock solutions (i.e. 100 µl of **reconstitute BSA** + 900 µl PBS buffer or DW).

2-2: Prepare standard solutions for macro assay method (serial dilution):

1- Number 7 vials from 7 to 1. You can use a "1 mgr/ml BSA stock solution" as **number 7**.

2- Add 500 ul of PBS to vials number 6-1.

3- Transfer 500 ul of vial 7 (1000 ugr/ml) to vial 6 and mix well by pipetting or vortexing.

4- Transfer 500 ul of vial 6 (500 ugr/ml) to vial 5 and mix well by pipetting or vortexing. Repeat this step for vials number 4, 3 and 2 and then throw away 500 ul of vial 2. Vial 1 is as blank.

Now you have standard solutions of BSA for macro assay that their concentrations are shown in table 2.

Table2) **Standard solutions for macro assay method**

No.	Concentration BSA [µg/ml]	Final volume [µl]	Required volume for test [µl]
1	0	500	20
2	31.25	500	20
3	62.5	500	20
4	125	500	20
5	250	500	20
6	500	500	20
7	1000	500	20

2-3: Prepare standard solutions for micro assay method (serial dilution):

1- Number 6 vials from 6 to 1.

2- Add 950 ul of PBS to vial 6 and 500 ul of PBS to vials number 5 to 1.

3- Transfer 50 ul BSA solution from **"1 mgr/ml BSA stock solution"** and mix well.

4- Transfer 500 ul of vial 6 (50 ugr/ml) to vials number 5, mix well and repeat this step for vial 4, 3 and 2. At the end hrowaway 500 ul of vial 2. Vial number 1 used as blank.

Now you have standard solutions of BSA for micro assay that their concentrations are shown in table 3.

Table 3) **Standard solutions for micro assay method**

No.	Concentration BSA [µg/ml]	Final volume [µl]	Required volume for test [µl]
1	0	500	20
2	3.1	500	20
3	6.25	500	20
4	12.5	500	20
5	25	500	20
6	50	500	20

3. Protein quantification procedure

3-1. Add 180 ul of **1X working solution DNAbiotech** bradford assay reagent to each well of ELISA plate.

3-2. Apply 20 ul of standard solutions or samples to each well and mix well by pipetting.

Note 1. In the micro assay method (or low concentration protein sample), it is recommended that pipetting carefully and mix the reagents completely.

Note 2. Use your background solution (e.g lysis buffer or …) as blank.

3-3. Incubate the plate at room temperature for 5 min.

3-4. Measure the absorbance (Optical Density, OD) at or near 595 nm with a plate reader.

4. Calculation of the protein concentration

4-1. If the spectrophotometer or micro plate reader was not zeroed with the blank, then average the blank values and subtract the average blank value from the standard and unknown sample values.

4-2. Create a standard curve by plotting the 595 nm values (y-axis) versus their concentration in µg/ml (x-axis). **Determine the unknown sample concentration using the standard curve.** If the samples were diluted, **adjust the final concentration of the unknown samples by multiplying by the dilution factor used**.

4-3. Standard curve examples for the macro assay method and the micro assay procedure are listed in Figures 1 and 2, respectively.

Note: The data below should not be used as a replacement of a calibration curve. The absorbance of the BSA reference solutions in each assay will differ from those presented here.

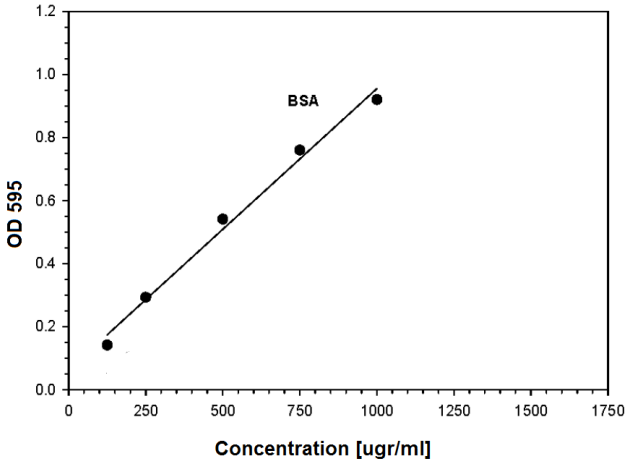

Fig.1. Typical standard curves by the macro assay method

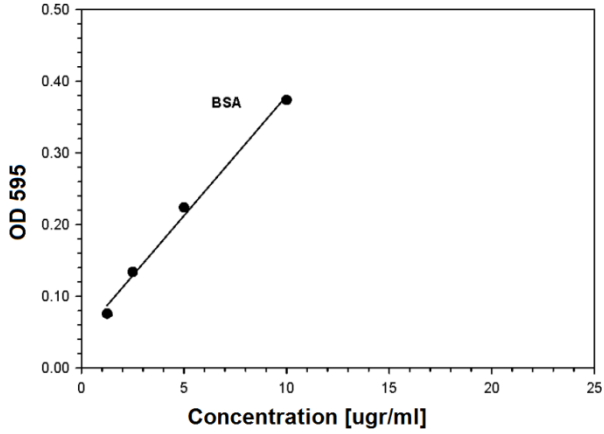

Fig.2. Typical standard curves by the micro assay method

Formula:

Concentration: the values obtained for the standard curved × dilution factor

Example for sample dilution:

1:20 dilution: 10 µl sample + 190 µl diluent
1:40 dilution: 5 µl sample + 195 µl diluents

5. Strategies for minimizing the effects of interfering substances

The effects of interfering substances in the Coomassie Assay may be overcome by several methods

- Remove the interfering substance by dialysis or desalting.
- Dilute the sample until the substance no longer interferes.
- Precipitate proteins with acetone or trichloroacetic acid (TCA). Upon precipitation the liquid containing the substance that interfered is discarded and the protein pellet is solubilized in a small amount of ultrapure water or directly in the Coomassie Reagent.

Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).

6. Effects of Coexisting Substances:

The Bradford method is comparatively resistant to the effects of reducing agents, but high concentrations of surfactants may affect measurement. The concentrations at which measurement is recommended are listed here:

Acetone, 10%
Acetonitrile, 10%
ASB-14, 0.025%
Ascorbic acid, 50 mM
Bis-Tris, pH 6.5, 0.2 M
β-mercaptoethanol, 1 M
Calcium chloride, 40 mM
CHAPS, 10%
CHAPSO, 10%
Deoxycholic acid, 0.2%
DMSO, 5%
Dithioerythritol (DTE), 10 mM
Dithiothreitol (DTT), 10 mM
Eagle's MEM
Earle's salt solution
MES, 0.1 M
Methanol, 10%
Modified Dulbecco's PBS
MOPS, 0.1 M
NAD, 2 mM
Nonidet P-40, 0.25%
Octyl β-glucoside, 0.5%
Octyl β-thioglucopyranoside, 1%
PBS 1x
Phenol Red, 0.5 mg/ml
PIPES, 0.2 M
PMSF, 2 mM
Potassium chloride, 2 M
Potassium phosphate, 0.5 M
SB 3–10, 0.1%
SDS, 0.025%
Sodium acetate, pH 4.8, 0.2 M
Sodium azide, 0.5%
Sodium bicarbonate, 0.2 M
Sodium carbonate, 0.1 M
Sodium chloride, 2.5 M
Ammonium sulfate, 1 M
Ampholytes, 3–10, 0.5%
Sodium citrate, pH 4.8 or 6.4 Magnesium, 0.2 M
Sodium hydroxide, 0.1 M
Sodium phosphate, 0.5 M
Sucrose, 10%
TBP, 5 mM
TBS (25 mM Tris, 0.15 M NaCl, pH 7.6), 0.5x
TCEP, 20 mM
Thio-urea, 1 M
Tricine, pH 8, 50 mM
Triethanolamine, pH 7.8, 50 mM
Tris, 1 M
Tris-glycine (25 mM Tris, 192 mM glycine)
Tris-glycine-SDS, (25 mM Tris, 192 mM glycine, 0.1% SDS), 0.5x
Triton X-100, 0.05%
Tween 20, 0.01%
Urea, 4 M
EDTA, 0.2 M
EGTA, 0.2 M
Ethanol, 10%
Glucose, 20%
Glycerol, 5%
Glycine, 0.1 M
Guanidine-HCl, 2 M
Hank's salt solution
HCl, 0.1 M
HEPES, 0.1 M
Imidazole, 0.2 M
Chloride, 1 M

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- ✓ Cloning and expression of different recombinant peptides
- ✓ Gene, Primer and peptide synthesizing
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- ✓ Production of column based DNA extraction kits.
- ✓ Production of secondary antibodies (goat anti mouse, anti rabbit and anti human antibodies, HRP conjugated).
- ✓ Taq polymerase and PFU master mix
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- ✓ And ….

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