Cat. # T9310A

For Research Use

TaKaRa

TaKaRa Bradford Protein Assay Kit

Product Manual

v201308Da

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I. Description

TaKaRa Bradford Protein Assay Kit can quickly measure protein concentration within a range of 1-1000 μg/ml using a simple protocol. The Bradford assay is based on measurement of the absorbance shift from 465 nm to 595 nm (brown to blue) that occurs upon Coomassie dye binding with protein. This change is proportionate to the amount of protein in solution, making it possible to assay protein concentration by measuring absorbance at 595 nm.

The protein/dye complex is stable for 5 to 60 minutes after the start of the reaction. It is possible to perform measurement with this kit even in the presence of a reducing agents, but surfactants in the protein solution may result in inaccurate measurements. Additionally, coloration differs greatly depending on the type of protein.

When the standard protocol is followed, this kit contains sufficient reagents for 500 assays when a 1 ml reaction volume is used and 2500 assays when a 200 μl reaction volume is used. When the low-concentration protocol is used, the kit can perform 1000 assays with a 1 ml reaction volume and 5000 assays with a 200 μl reaction volume.

II. Contents

1. Bradford Dye Reagent 250 ml x 2
2. BSA Standard Solution (2 mg/ml)* 1 ml x 10

* : The BSA standard solution contains 0.9% NaCl and 0.05% NaN_3 as stabilizers.

III. Materials Required but not Provided

- Spectrophotometer and compatible 1 ml cuvette
- Microplate reader and compatible microplate
- Benchtop centrifuge
- Microtubes (2 ml or 1.5 ml)
- Spectrophotometer or microplate reader

IV. Storage 4℃

V. Precautions for Use

The following are precautions for the use of this product. Please read them before use.

1. Bring the Bradford Dye Reagent to room temperature before use. Invert the tube 3-5 times to mix immediately before use. Avoid vigorous shaking.
2. Before use, bring the BSA Standard Solution to room temperature or place in a 20-50℃ water bath. After warming, vortex or tap lightly to mix well then briefly spin down.
3. Deionized water, 0.9% NaCl, or PBS may be used for dilution of the standard solution and samples.
4. The protein/dye complex may precipitate after the reaction. Be sure to mix the reaction solution uniformly before measuring absorbance.
5. 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.
6. 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.

VI. Protocol

VI-a. Standard Protocol (range: 25 - 1,000 μg/ml; 1 ml reaction)

1. Prepare dilutions of the BSA standard solution as shown below. Deionized water, 0.9% NaCl, or PBS may be used to dilute the BSA standard solution and the sample.

2 mg/ml BSA standard (μl)	Diluent (μl)	Final concentration of BSA (μg/ml)
50	50	1,000
30	50	750
20	60	500
20	140	250
10	150	125
5	395	25
0	100	0 (Blank)

2. Dispense 20 μl of each dilution of the BSA standard solution or the sample (preparing serial dilutions if necessary) into 1.5 ml microtubes. Perform at least 2 replicate measurements (n=2) for each standard dilution and sample.
3. Add 1 ml of pre-warmed Bradford Dye Reagent to each tube and mix well. Incubate for 5 minutes in a 25℃ water bath or at room temperature (~25℃).
4. Measure absorbance at 595 nm. Measure absorbance within 1 hour of the reaction.
5. Subtract the average blank measurement from all other individual standard and unknown sample replicates. Determine the protein concentration of the sample using a standard curve generated from the dilutions of the BSA standard.

VI-b. Standard Protocol (range: 25 - 1,000 μg/ml; 200 μl reaction)

1. Prepare dilutions of the BSA standard solution as in VI-a-1.
2. Dispense 4 μl of each dilution of the BSA standard solution or the sample (preparing serial dilutions if necessary) into the wells of the microtiter plate. Perform at least 2 replicate measurements (n=2) for each standard dilution and sample.
3. Add 200 μl of pre-warmed Bradford Dye Reagent to each well and mix. Incubate for 5 minutes at a room temperature (~25℃).
4. Use a microplate reader to measure absorbance at 595 nm. Measure absorbance within 1 hour of the reaction.
5. Subtract the average blank measurement from all other individual standard and unknown sample replicates. Determine the protein concentration of the sample using a standard curve generated from the dilutions of the BSA standard.

VI-c. Low-Concentration Protocol (range: 1-25 μg/ml; 1 ml reaction)

1. Mix 50 μl of the BSA standard solution (2 mg/ml) with 950 μl of diluent and mix well to prepare a 0.1 mg/ml BSA standard solution.

   Prepare 2 sets of the dilutions of BSA standard solution as shown below 1.5 ml microtubes (7 types x 2 sets = 14 microtubes). Deionized water, 0.9% NaCl, or PBS may be used to dilute the BSA standard solution and the samples.

0.1 mg/ml BSA standard solution (μl)	Diluent (μl)	Final concentration of BSA (μg/ml)
125	375	25
100	400	20
75	425	15
50	450	10
25	475	5
12.5	487.5	2.5
0	500	0 (Blank)

2. Dispense 500 μl of the sample (preparing serial dilutions if necessary) into 1.5 ml microtubes. Perform at least 2 replicate measurements (n=2) for each standard dilution and sample.
3. Add 0.5 ml of pre-warmed Bradford Dye Reagent to each tube and mix well. Incubate for 5 minutes in a 25℃ water bath or at room temperature (~25℃).
4. Measure absorbance at 595 nm within 1 hour of the reaction.
5. Subtract the average blank measurement from all other individual standard and unknown sample replicates. Determine the protein concentration of the sample using a standard curve generated from the dilutions of the BSA standard.

VI-d. Low-Concentration Protocol (range: 1-25 μg/ml; 200 μl reaction)

1. Prepare dilutions of BSA standard solution as in VI-c-1.
2. Dispense 100 μl of each dilution of the BSA standard or the sample (preparing serial dilutions if necessary) into the wells of the microtiter plate. Perform at least 2 replicate measurements (n=2) for each standard dilution and sample.
3. Add 100 μl of pre-warmed Bradford Dye Reagent to each well and mix. Incubate for 5 minutes at a room temperature (~25℃).
4. Use a microplate reader to measure absorbance at 595 nm. Measure absorbance within 1 hour of the reaction.
5. Subtract the average blank measurement from all other individual standard and unknown sample replicates. Determine the protein concentration of the sample using a standard curve generated from the dilutions of the BSA standard.

VII. Appendix

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 with this kit is unaffected are shown in Table 1.

Table 1. Permissible Concentrations of Various Reagents under the Standard Protocol

Substance	Permissible Concentration of the Coexisting Substance
Salts/ Buffers	
Ammonium sulfate	1 M
Borate pH9.5	50 mM
Calcium chloride	10 mM
Glycine	100 mM
Guanidine-HCl	3.5 M
HEPES, pH7.5	100 mM
Imidazole, pH7.0	200 mM
KPB, pH7.0	100 mM
Magnesium chloride	100 mM
MES, pH6.1	100 mM
MOPS, pH7.2	100 mM
NaPB, pH7.0	100 mM
Nickel chloride	10 mM
PBS	undiluted
PIPES, pH6.8	500 mM
Sodium acetate, pH5.0	600 mM
Sodium azide	0.5%
Sodium chloride	5 M
Sodium citrate, pH6.4	200 mM
Tricine, pH8.0	100 mM
Tris-HCl, pH8.0	2 M
Zinc chloride	10 mM
Detergents	
Brij®-35	0.125%
CHAPS	5%
Nonidet P-40 (NP-40)	0.1%
Triton® X-100	0.125%
Tween®-20	0.1%
SDS	0.015%

Substance	Permissible Concentration of the Coexisting Substance
Chelating agents	
EDTA	100 mM
EGTA	50 mM
Reducing agents	
Cysteine	10 mM
Dithiothreitol (DTT)	100 mM
Glucose	1 M
2-Mercaptoethanol	1 M
Organic solvents	
Acetone	10%
DMSO	10%
Ethanol	10%
Methanol	10%
Misc. Reagents	
Glycerol	50%
Hydrochloric Acid	100 mM
PMSF	1 mM
16S, 23S rRNA	1 mg/ml
Sodium Hydroxide	100 mM
Streptomycin sulfate	20%
Tryptophan	1 mM
Urea	6 M

Effects Protein Type

Coomassie dye binds primarily to the basic amino acids and aromatic amino acids in the protein (arginine residues in particular). The degree of coloration will differ based on the type of protein. Figure 1 shows standard curves for the conventionally used standards BSA (Bovine Serum Albumin) and BGG (Bovine Gamma Globulin). Table 2 shows the ratio of coloration of 15 typical proteins when compared with BSA.

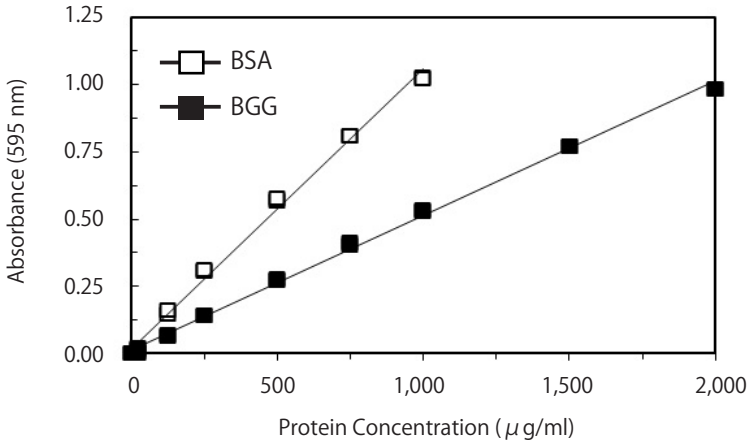

Fig. 1: Standard Curves for BSA and BGG

Table 2. Coloration Ratios of Various Proteins to BSA

Protein	Ratio*
Albumin, bovine serum (BSA)	1.00
Alcohol Dehydrogenase, *Saccharomyces cerevisiae*	0.64
Aldolase, rabbit muscle	0.80
Carbonic Anhydrase, bovine erythrocytes	0.89
a-Chymotrypsin, bovine pancreas	0.52
Cytochrome C, bovine heart	1.31
Gamma globulin, bovine (BGG)	0.51
Hemoglobin, bovine	1.01
IgG, rabbit	0.40
IgG, mouse	0.58
Insulin, human	0.84
Lysozyme, chicken egg white	0.73
Myoglobin, equine skeletal muscle	1.15
Ovalbumin, chicken egg white	0.68
Transferrin, human	0.79

* Ratio = (Mean net absorbance of individual proteins) / (Mean net absorbance of BSA)

VIII. Related Products

TaKaRa BCA Protein Assay Kit (Cat. # T9300A)