

Protein Determination Reagents



Protein Quantification Reagents

Bicinchoninic Acid (BCA) Assay

New BCA Protein Assay Kit

1kit [B7124]

Advantages

- The Cu^{2+} -BCA complex, which shows an absorbance maximum at around 480 nm, is reduced by proteins to form a Cu^+ -BCA complex. This reaction shifts the absorbance maximum to 562 nm in a manner linear to protein concentration, thus enabling accurate quantification of protein concentration.
- High-sensitivity protein quantification.
- Excellent compatibility with detergents.
- One kit enables quantification of up to 1,250 wells.*

(*Compatible with 96-well plate format for convenient, high-throughput analysis)



Application

1. Prepare a dilution series using a BSA standard solution.
2. Mix Reagent A and Reagent B at a ratio of 50:1.
3. Apply this mixed reagent to test samples, BSA standards, and distilled water blank at the proportions shown in Table 1.
4. Incubate at 37 °C for 30 minutes.
5. Measure absorbance at a wavelength between 540-570 nm (562 nm if possible).
6. Generate a calibration curve by subtracting the distilled water value from that of the BSA standards, and use to calculate sample protein concentrations.

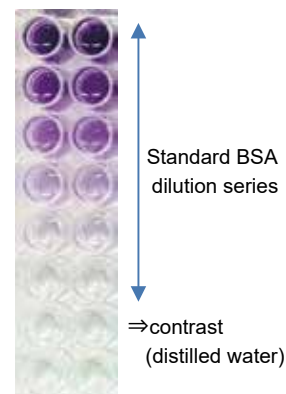
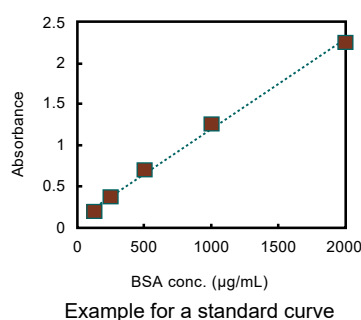
Table 1. Volumes for test tube or microplate assay using B7124

	test tube	microplate
Measurement Range	0.1 mg/mL – 2 mg/mL	0.1 mg/mL – 2 mg/mL
Sample Solution or Protein Standard	50 μL	25 μL
B7124 (After Mixing 50:1 Reagent)	1 mL	200 μL

This product requires the standard protein solution (such as BSA).

Example for use: in a microplate

1. Prepare a two-fold serial dilution of the BSA standard solution starting from 2.0 mg/mL.
2. Mix Reagent A and Reagent B at a 50:1 ratio. Add 25 μL of each standard solution and 200 μL of the mixed reagent to the plate.
3. Incubate at 37 °C for 30 minutes to allow the reaction to proceed.
4. Measure the absorbance at 560 nm after incubation and generate a calibration curve.



Compatible substance concentrations in protein sample of B7124

Substances at the following concentrations in the sample solutions do not affect the reaction results.

Buffers		Salts		Detergents		Additives	
HEPES	100 mM	Ammonium Sulfate	150 mM	CHAPS	5 %	Glycerol	30 %
MES	100 mM	Calcium Chloride	15 mM	Nonidet P-40	10 %	Imidazole	50 mM
MOPS	100 mM	Sodium Chloride	1.25 M	SDS	5 %	Sodium Azide	0.30 %
PIPES	100 mM	Solvents		Triton X-100	7.50 %	pH Adjusters	
Sodium Citrate	100 mM	DMSO	50 %	Tween-20	5 %	Hydrochloric Acid (HCl)	200 mM
Tricine	25 mM	Acetone	50 %	2-Mercapto-ethanol	0.5 mM	Sodium Hydroxide (NaOH)	150 mM
Tris	50 mM	Ethanol	50 %	DTE	0.05 mM	Chelating Agents	
Sodium Acetate pH5.2	400 mM	Methanol	50 %	Urea	4 M	EDTA	15 mM

Bradford Protein Assay

Bradford Assay Solution (Ready-to-use) [for Protein determination]

500mL [B5702]

Advantages

- Applicable to determine the amounts of proteins in test samples, because the dye containing coomassie brilliant blue G-250 binds proteins and the absorption maximum of the dye shifts from 465 nm to 595 nm in a linear manner with an increase in the quantity of proteins.
- One-component ready-to-use solution
- Absorbance can be measured only 5 minutes after the reaction starts.
- Low concentration of protein (1.0 - 25 µg/mL) can be measured.

Application

1. Prepare standard protein solutions with a series of dilutions.
2. Mix B5702 with unknown protein samples, standard protein solutions and distilled water according to Table 2.
3. Incubate for 5 minutes at room temperature.
4. Measure absorbance at 600 nm.
5. Prepare a standard curve by plotting the absorbance data measured in step 4 after subtracting from blank absorbance (distilled water), and calculate the amount of protein in test samples.

Table 2. Volumes for test tube or microplate assay using B5702

	test tube	microplate	micro assay
Measurement Range	0.1 - 1.0 mg/mL	0.1 - 1.0 mg/mL	0.1 - 25 µg/mL
Sample Solution or Protein Standard	20 µL	4 µL	500 µL
B5702	1 mL	200 µL	500 µL

This product requires the standard protein solution (such as BSA).

Compatible substance concentrations in protein sample of B5702

Substances at the following concentrations in the sample solutions do not affect the reaction results.

Buffers		Salts		Solvents		Denaturants	
Glycine	100 mM	(NH ₄) ₂ SO ₄	1 M	Acetone	10 %	DTT	100 mM
Tris	2 M	KCl	1 M	DMSO	10 %	Glutathione	1 mg/mL
HCl	100 mM	MgCl ₂	50 mM	Ethanol	10 %	2-Mercapto-ethanol	1 M
HEPES	100 mM	CaCl ₂	10 mM	Methanol	10 %	Guanidine Hydrochloride	1 M
MES	100 mM	NiCl ₂	10 mM	Glycerol	10 %	Urea	200 mM
MOPS	100 mM	ZnCl ₂	10 mM				
PIPES	100 mM	NaCl	2 M	Detergents		Chelating Agents	
Glucose	1 M	NaOH	100 mM	SDS	0.05 %	EDTA	100 mM
Sucrose	25 %	NaH ₂ PO ₄	500 mM	Triton X-100	0.10 %	EGTA	10 mM
Fructose	1 M	NaN ₃	50 %	Tween-20	0.10 %	Sodium Citrate	200 mM

Pyrogallol Red-Molybdate Protein Assay

Pyrogallol Red (Ready-to-use) [for Protein determination]

100mL [P2575]

Advantages

- **Applicable to determine the amounts of proteins in test samples, because the dye containing pyrogallol red-molybdate complex binds proteins and the absorption maximum of the dye shifts from 480 nm to 600 nm in a linear manner with an increase in the quantity of proteins.**
- **One-component ready-to-use solution.**
- **Very little staining of cuvettes which can be washed with water alone after use.**

Application

1. Prepare standard protein solutions with a series of dilutions.
2. Mix **P2575** with unknown protein samples, standard protein solutions and distilled water according to Table 3.
3. Incubate for 30 minutes at room temperature.
4. Measure absorbance at 600 nm.
5. Prepare a standard curve by plotting the absorbance data measured in step 4 after subtracting from blank absorbance (distilled water), and calculate the amount of protein in test samples.

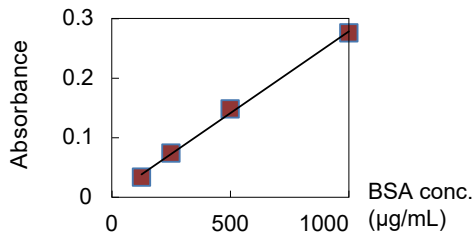
Table 3. Volumes for test tube or microplate assay using **P2575**

	test tube	microplate	micro assay
Measurement Range	0.1 - 1.0 mg/mL	0.1 - 1.0 mg/mL	0.1 - 25 µg/mL
Sample Solution or Protein Standard	20 µL	4 µL	500 µL
P2575	1 mL	200 µL	500 µL

This product requires the standard protein solution (such as BSA).

Example for use: in a microplate

1. Prepare four dilution series of standard protein solutions from the concentration at 1000 µg/mL by doubling dilution.
2. Mix 200 µL of **P2575** with 10 µL each of a protein sample at an unknown concentration, the standard protein solution and distilled water in a 96-well microplate.
3. Incubate for 30 minutes at room temperature, measure absorbance at 600 nm, and prepare a standard curve.



Example for a standard curve



Standard BSA dilution series

⇒ contrast (distilled water)

⇒ unknown sample

Example for a reaction

Compatible substance concentrations in protein sample of **P2575**

Substances at the following concentrations in the sample solutions do not affect the reaction results.

Buffers		Salts		Solvents		Denaturants	
Glycine	100 mM	(NH ₄) ₂ SO ₄	1 M	Acetone	10 %	DTT	100 mM
Tris	2 M	KCl	1 M	DMSO	10 %	Glutathione	1 mg/mL
HCl	200 mM	MgCl ₂	50 mM	Ethanol	10 %	2-Mercapto-ethanol	1 M
HEPES	100 mM	CaCl ₂	10 mM	Methanol	10 %	Guanidine Hydrochloride	1 M
MES	100 mM	NiCl ₂	10 mM	Glycerol	10 %	Urea	3 M
MOPS	100 mM	ZnCl ₂	10 mM	Detergents Chelating Agents			
PIPES	100 mM	NaCl	2 M				
Tricine	100 mM	NaOH	100 mM	SDS	0.10 %	EDTA	100 mM
Imidazole	200 mM	NaH ₂ PO ₄	500 mM	Triton X-100	0.10 %	EGTA	10 mM
Glucose	1 M	NaN ₃	0.50 %	Tween-20	0.10 %	Sodium Citrate	200 mM
Sucrose	25 %						
Fructose	1 M						

Comparison of Protein Quantification Reagents

	BCA Assay [B7124]	Pyrogallol Red-Molybdate Assay [P2575]	Bradford Assay [B5702]
Main Advantages	Minimal variation in measurement across different protein types	Stable absorbance after color development	Rapid reaction capable of quick measurement
Storage Temperature	Room Temperature	Refrigeration (<4°C)	Refrigeration (<4°C)
Tolerant to certain interfering substances, such as:	Detergents, salts, and buffer components	Reducing agents and salts	Reducing agents and salts
Susceptible to certain interfering substances, such as:	Reducing agents and copper ion chelators	Detergents	Detergents, strong acids, and strong bases

Related Products

Standard Solution of Albumin from Bovine Serum

5mL **[T3796]**

Bicinchoninic Acid Disodium Salt [for Protein Research]

5g **[B5838]**

Pyrogallol Red [for Protein Research]

1g **[P1976]**

Gel Staining Reagents

Silver Staining

Silver Stain Kit [for Electrophoresis]

1kit [I1309]

Silver staining is a commonly-used method for the detection of proteins and DNA in polyacrylamide gels after electrophoresis. In this method, silver ions are bound to proteins and DNA present in the gel and reduced, resulting in stained bands. Silver staining is more sensitive than Coomassie Brilliant Blue (CBB) staining; it can detect down to nanogram amounts of protein.

Advantages

- **Rapid Staining** (approx. 1 hour)
- **Highly Sensitive** (Several ng)
- **Odorless** - ammonium ion-free
- **Safe** - does not produce explosive silver amides



Usage Example

1. Prepare Fixing Solution, Staining Solution, Developer Solution, and Stop Solution by diluting the supplied solutions 100-fold.
2. In a clean tray, submerge the gel in Fixing Solution, and allow to incubate with shaking for 10 minutes.
3. Remove Fixing Solution, and wash gel in deionized water with shaking for 10 minutes. (Repeat a total of three times)
4. Remove deionized water and replace with Staining Solution. Incubate with shaking for 5 minutes.
5. Remove Staining Solution and replace with deionized water. Incubate with shaking for 30 seconds.
6. Remove deionized water and replace with Developer Solution. Incubate with shaking for 30 seconds.
7. Replace old Developer Solution with fresh solution. Incubate with shaking until developed bands appear.
8. Remove Developer Solution and replace with Stop Solution. Incubate with shaking for 10 minutes.
9. Remove Stop Solution, and wash gel a total of three times with deionized water, incubating with shaking for 5 minutes each wash.

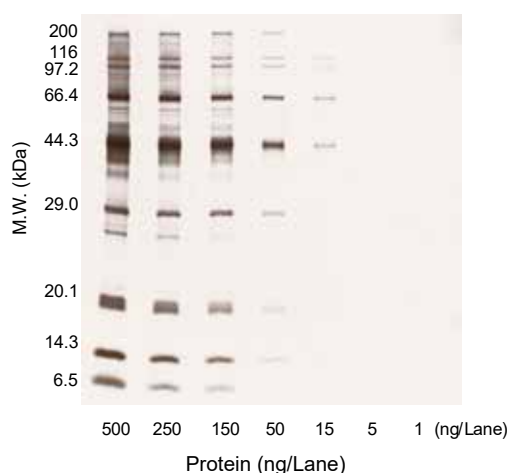


Figure A. Protein molecular weight markers were diluted, run on an acrylamide gel, and stained

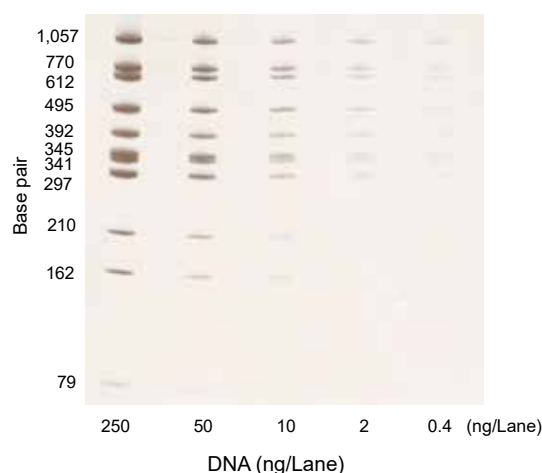


Figure B. DNA molecular weight markers at each concentration were diluted, run on an acrylamide gel, and stained

Negative Staining

Gel Negative Stain kit [for Electrophoresis]

1kit [G0615]

Negative staining is a detection method for SDS-PAGE-separated proteins in which only regions of gel not containing proteins are stained white, while protein-containing regions remain transparent. After staining, the gel can be easily destained with a destaining solution and transferred to a membrane.

Advantages

- Rapid Staining (approx. 20 minutes)
 - Highly Sensitive
 - Allows the Use of Destained Gels in Downstream Experiments
 - Stains 20* Gels per Kit
- *90 x 90 x 1 mm gel



Directions for Use

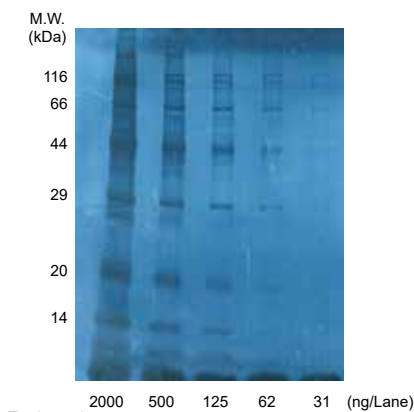
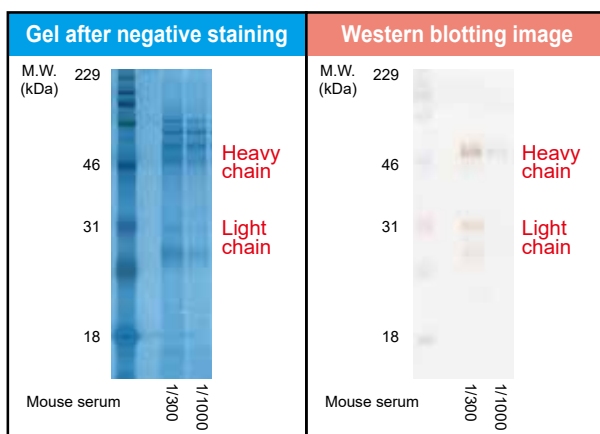


Figure C. Gel stained by the method described at right.

1. Place the post-SDS-PAGE gel in a tray containing enough deionized water to cover the gel and shake for 10 minutes.
2. Discard the deionized water, add enough solution A (diluted 10 times with deionized water) to cover the gel and shake for 5 minutes.
3. Submerge gel in deionized water for 10 seconds to wash. Repeat three times.
4. Transfer the gel to a new tray, add enough solution B (diluted 10 times with deionized water) to cover the gel and shake for 1 minute to develop color.

Directions for Use (preparation of gel for Western Blotting)



1. Place the stained and photographed gel in a tray containing solution C diluted 10-fold with deionized water.
 2. Shake the gel until the color is removed.
 3. Discard solution C, add enough deionized water to cover the gel and wash for 30 seconds. Repeat three times.
 4. Transfer the washed gel to a membrane (PVDF).
- Primary Antibodies: **Goat Anti-Mouse IgG Biotin** [G0387]
 Secondary Antibodies: **Streptavidin HRP Conjugate** [S0972]
 Chromogenic Substrate: **3,3'-Diaminobenzidine (DAB)** [D3756]

CBB Staining

Coomassie Brilliant Blue G-250 (Ready-to-use solution) [for Electrophoresis] 500mL [C3488]

Application

1. After electrophoresis, wash the gel with deionized water for 5 minutes three times.
2. Remove the water used for washing, add **C3488** till the gel is soaked, and stain the gel for 1 hour while shaking gently at room temperature.
3. Remove the staining solution, destain the gel with deionized water for 1 hour and check it.
4. If the background is high, destain the gel with deionized water overnight at room temperature.

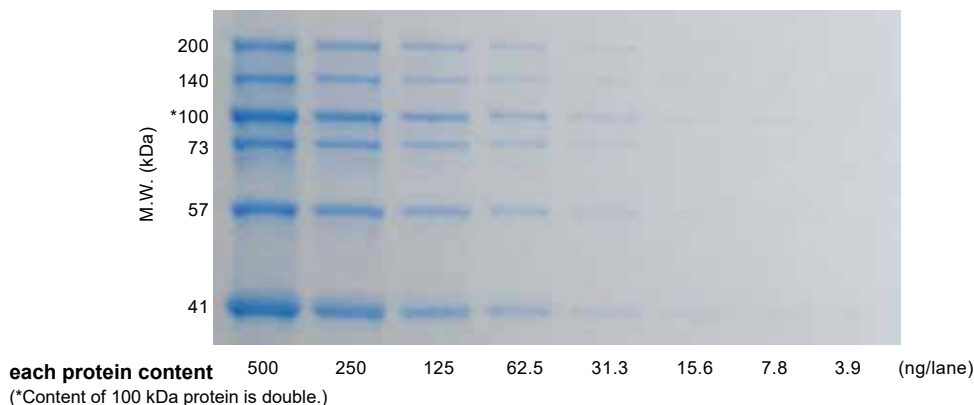


Figure D. Proteins stained by the above method (destained overnight)

Comparison of Silver Staining, Negative Staining and CBB Staining

	Time	Detection sensitivity	Advantages
Silver Stain [I1309]	~1 hour	Several ng	Highly sensitive detection method with ample track record Able to detect both protein and DNA
Negative Stain [G0615]	15 - 30 minutes	Several ng	Short staining time Stained gels can be used for Western blotting, etc.
CBB Stain [C3488]	2 hours - over night	Several µg	Easy-to-use, simple protocol Resultant bands are quantifiable

Reagents for Protein Staining and Others

Acid Black 1 (= Amido Black 10B)	5g [A2097]
Acid Red 112 (= Ponceau S)	1g / 5g [A2256]
Coomassie Brilliant Blue G-250	5g [B3193]
Coomassie Brilliant Blue R-250	5g [B3194]
Fast Green FCF	5g [F0718]
Sodium Deoxycholate	25g [D1820]
6-Aminohexanoic Acid	5g / 25g [A2255]

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