

# Protein Determination Reagents

## Pyrogallol red-molybdate protein assay

### Pyrogallol Red (Ready-to-use solution) [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 1.
- 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 #4) after subtracting from blank absorbance (distilled water), and calculate the amount of protein in test samples.

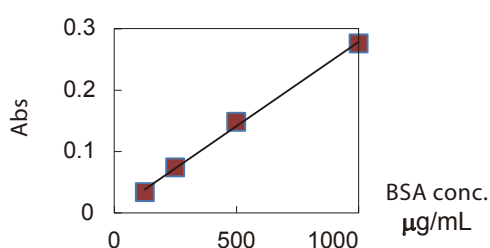
Table 1 : Volume for test tube or micro plate assay

Assay	test tube	micro plate
Measurement range	0.1 -1.0 mg/mL	0.1 -1.0 mg/mL
Sample solution or *protein standard	50 $\mu$ L	10 $\mu$ L
<b>P2575</b>	1 mL	200 $\mu$ L

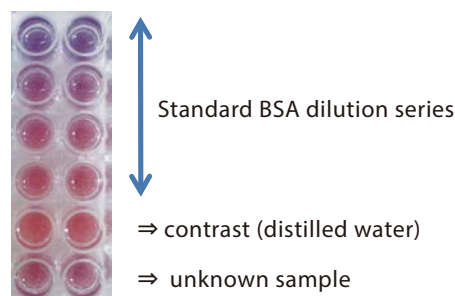
\*This product requires the standard protein solution (such as BSA).

#### Example for use: in a microplate

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



Example for a standard curve



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

## Related Products

<b>Pyrogallol Red</b> [for Protein Research]	1g [P1976]
<b>Streptomycin Sulfate</b> [for Protein Research]	5g / 25g [S0834]
<b>Acrylamide Monomer</b> [for Electrophoresis]	25g / 500g [A1132]
<b>Acid Black 1</b> [for Electrophoresis]	5g [A2097]
<b>Ammonium Peroxodisulfate</b> [for Protein Research]	5g / 25g [A2098]
<b>Coomassie Brilliant Blue G-250</b> [for Electrophoresis]	5g [B3193]
<b>Coomassie Brilliant Blue R-250</b> [for Electrophoresis]	5g [B3194]
<b>Bromophenol Blue Sodium Salt</b> [for Electrophoresis]	1g [B3195]
<b>Sodium Deoxycholate</b> [for Electrophoresis]	25g [D1820]
<b>DL-Dithiothreitol</b> [for Electrophoresis]	1g / 5g [D3647]
<b>Glycerol</b> [for Electrophoresis]	1g [G0316]
<b>Glycine</b> [for Electrophoresis]	25g / 500g [G0317]
<b>N,N'-Methylenebisacrylamide</b> [for Electrophoresis]	25g / 100g [M0506]
<b>2-Mercaptoethanol</b> [for Electrophoresis]	5g / 25g [M1948]
<b>Sodium Dodecyl Sulfate</b> [for Electrophoresis]	25g / 500g [S0588]
<b>N,N,N',N'-Tetramethylethylenediamine</b> [for Electrophoresis]	5g / 25g [T2515]
<b>Tris(hydroxymethyl)aminomethane</b> [for Electrophoresis]	25g / 500g [T2516]

## 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 #4) after subtracting from blank absorbance (distilled water), and calculate the amount of protein in test samples.

Table 2 : Volume for test tube or micro plate assay

Assay	test tube	micro plate	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
<b>B5702</b>	1 mL	200 µL	500 µL

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

#### Compatible substance concentrations in protein sample

Buffers		Salts		Solvents		Denaturants	
Substance	Conc.	Substance	Conc.	Substance	Conc.	Substance	Conc.
Glycine	100 mM	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1 M	Acetone	10 %	DTT	100 mM
Tris	2 M	KCl	1 M	DMSO	10 %	Glutathione	1 mg / mL
HCl	100 mM	MgCl <sub>2</sub>	50 mM	Ethanol	10 %	2-Mercaptoethanol	1 M
HEPES	100 mM	CaCl <sub>2</sub>	10 mM	Methanol	10 %	Guanidine-HCl	1 M
MES	100 mM	NiCl <sub>2</sub>	10 mM	Glycerol	10 %	Urea	3 M
MOPS	100 mM	ZnCl <sub>2</sub>	10 mM				
PIPES	100 mM	NaCl	2 M	Detergents		Chelating Agents	
Glucose	1 M	NaOH	100 mM	Substance	Conc.	Substance	Conc.
Sucrose	25 %	NaH <sub>2</sub> PO <sub>4</sub>	500 mM	SDS	0.05 %	EDTA	100 mM
Fructose	1 M	NaN <sub>3</sub>	0.50 %	Triton X-100	0.10 %	EGTA	10 mM
				Tween-20	0.10 %	Sodium citrate	200 mM

## CBB protein staining

### Coomassie Brilliant Blue G-250

(Ready-to-use solution) [for Electrophoresis]

500mL [C3488]

#### Advantages

- Usable for gel staining after electrophoresis
- One-component ready-to-use solution
- Free from methanol and acetic acid

#### 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 let the gel stain 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.

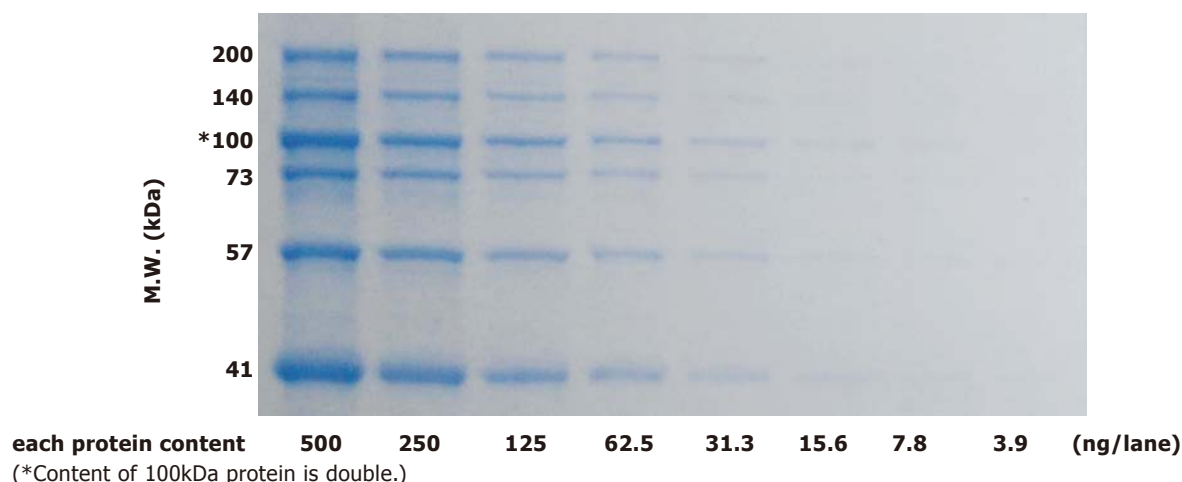


Figure. Proteins stained by the above method (destained overnight )

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