# Реагенты для определения белка

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

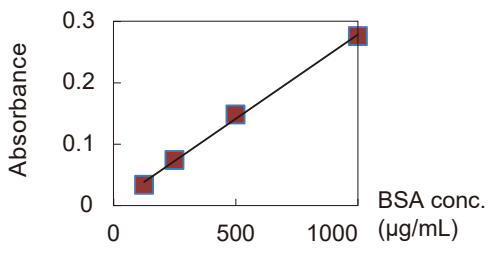
Table 1 : Volume for test tube or micro plate assay

| 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 µL | 10 µL |
| **P2575** | 1 mL | 200 µL |

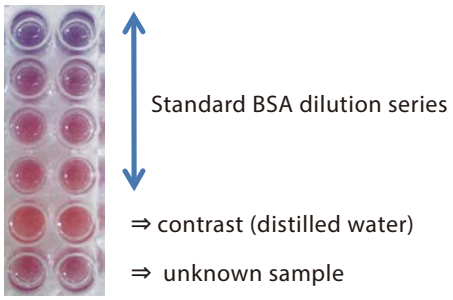
‡This product requires the standard protein solution (such as BSA).

### 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




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 | |
|---|---|
| Substance | Conc. |
| Glycine | 100 mM |
| Tris | 2 M |
| HCl | 200 mM |
| HEPES | 100 mM |
| MES | 100 mM |
| MOPS | 100 mM |
| PIPES | 100 mM |
| Tricine | 100 mM |
| Imidazole | 200 mM |
| Glucose | 1 M |
| Sucrose | 25 % |
| Fructose | 1 M |

| Chelating Agents | |
|---|---|
| Substance | Conc. |
| EDTA | 100 mM |
| EGTA | 10 mM |
| Sodium citrate | 200 mM |

| Salts | |
|---|---|
| Substance | Conc. |
| $(NH_4)_2SO_4$ | 1 M |
| KCl | 1 M |
| $MgCl_2$ | 50 mM |
| $CaCl_2$ | 10 mM |
| $NiCl_2$ | 10 mM |
| $ZnCl_2$ | 10 mM |
| NaCl | 2 M |
| NaOH | 100 mM |
| $NaH_2PO_4$ | 500 mM |
| $NaN_3$ | 0.50 % |

| Solvents | |
|---|---|
| Substance | Conc. |
| Acetone | 10 % |
| DMSO | 10 % |
| Ethanol | 10 % |
| Methanol | 10 % |
| Glycerol | 10 % |

| Denaturants | |
|---|---|
| Substance | Conc. |
| DTT | 100 mM |
| Glutathione | 1 mg/mL |
| 2-Mercaptoethanol | 1 M |
| Guanidine-HCl | 1 M |
| Urea | 3 M |

| Detergents | |
|---|---|
| Substance | Conc. |
| SDS | 0.10 % |
| Triton X-100 | 0.10 % |
| Tween-20 | 0.10 % |

## Related Products

**Standard Solution of Albumin from Bovine Serum** 5mL [T3796]
**Pyrogallol Red** [for Protein Research] 1g [P1976]
**Streptomycin Sulfate** [for Protein Research] 5g / 25g [S0834]
**Acrylamide Monomer** [for Electrophoresis] 25g / 500g [A1132]
**Acid Black 1** [for Electrophoresis] 5g [A2097]
**Ammonium Peroxodisulfate** [for Protein Research] 5g / 25g [A2098]
**Coomassie Brilliant Blue G-250** [for Electrophoresis] 5g [B3193]
**Coomassie Brilliant Blue R-250** [for Electrophoresis] 5g [B3194]
**Bromophenol Blue Sodium Salt** [for Electrophoresis] 1g [B3195]
**Sodium Deoxycholate** [for Electrophoresis] 25g [D1820]
**DL-Dithiothreitol** [for Electrophoresis] 1g / 5g [D3647]
**Glycerol** [for Electrophoresis] 1g [G0316]
**Glycine** [for Electrophoresis] 25g / 500g [G0317]
***N,N'*-Methylenebisacrylamide** [for Electrophoresis] 25g / 100g [M0506]
**2-Mercaptoethanol** [for Electrophoresis] 5g / 25g [M1948]
**Sodium Dodecyl Sulfate** [for Electrophoresis] 25g / 500g [S0588]
***N,N,N',N'*-Tetramethylethylenediamine** [for Electrophoresis] 5g / 25g [T2515]
**Tris(hydroxymethyl)aminomethane** [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 step 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 |
| **B5702** | 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 | $(NH_4)_2SO_4$ | 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-Mercaptoethanol | 1 M |
| HEPES | 100 mM | $CaCl_2$ | 10 mM | Methanol | 10 % | Guanidine-HCl | 1 M |
| MES | 100 mM | $NiCl_2$ | 10 mM | Glycerol | 10 % | Urea | 3 M |
| MOPS | 100 mM | $ZnCl_2$ | 10 mM | | | | |
| PIPES | 100 mM | NaCl | 2 M | **Detergents** | | **Chelating Agents** | |
| Glucose | 1 M | NaOH | 100 mM | Substance | Conc. | Substance | Conc. |
| Sucrose | 25 % | $NaH_2PO_4$ | 500 mM | SDS | 0.05 % | EDTA | 100 mM |
| Fructose | 1 M | $NaN_3$ | 0.50 % | Triton X-100 | 0.10 % | EGTA | 10 mM |
| | | | | Tween-20 | 0.10 % | Sodium citrate | 200 mM |

## CBB protein staining

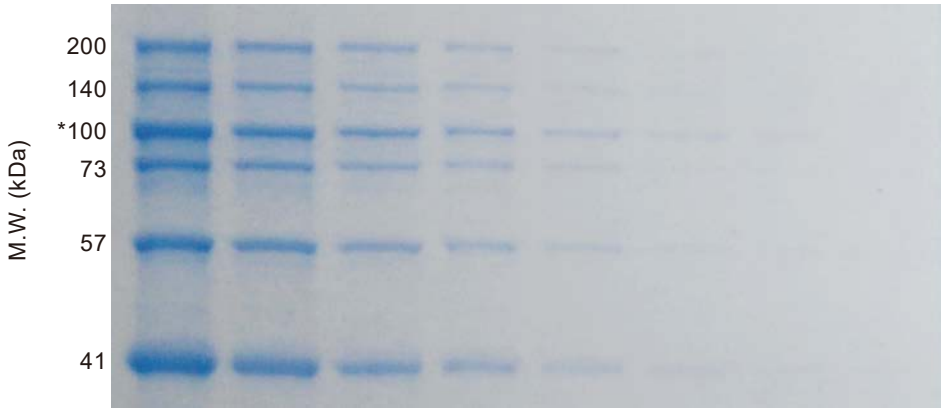
**Coomassie Brilliant Blue G-250**
**(Ready-to-use solution)** [for Electrophoresis] 500mL [C3488]

### 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.




each protein content 500 250 125 62.5 31.3 15.6 7.8 3.9 (ng/lane)
(*Content of 100kDa protein is double.)

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

## Bicinchoninic acid (BCA) assay

**Bicinchoninic Acid Disodium Salt** [for Protein Research] 5g [B5838]

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