# Instructions for ultra-low molecular weight protein Marker (3.3kD-20.1kD)

**Item No. :** PR1300

**Specification:** 15T (150μL)

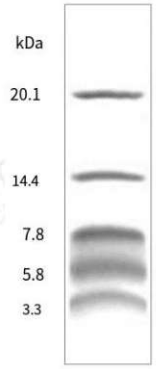
**Storage: Store** at -20 ° C, valid for one year.

**Product Introduction:**

This product contains 3 kinds of peptides and 2 kinds of low molecular weight protein composition, molecular weight range of 3. 3kD-20.1kD. It can be used to determine the molecular weight of polypeptides and small proteins on SDS-PAGE. This product is a freeze-dried powder of protein and polypeptide mixture, the content of each protein is about 15-22.5μg, equipped with a 1× loading buffer.

**Instructions for use (for reference only) :**

1. After opening, dissolve in 150μL 1× sample buffer, boiling water bath for 5 minutes, can be divided according to need, each tube 10μL, -20℃ storage, take one tube each time to use, to avoid repeated freeze-thaw.
2. Before use, take the small tube after subpacking and melt at room temperature, boiling water bath for 5 minutes can be used for sample electrophoresis, and 5 protein bands can be seen after dyeing with Coomas bright blue G-250 (see the diagram below).




**Gel configuration method:**

| | Separation gel | | | Sandwich glue | Concentrated glue |
|---|---|---|---|---|---|
| | 20% / 4.5 mL | 16.5% / 4.5 mL | 15.5% / 4.5 mL | 10%/2mL | 4%/2mL |
| 49.5%T 3%C | / | / | / | 0.407 mL | 0.160 mL |
| 49.5%T 6%C | 1.82 mL | 1.50 mL | 1.395 mL | / | / |
| Gel buffer | 1.50 mL | 1.50 mL | 1.50 mL | 0.667 mL | 0.496 mL |
| Glycerin | 0.48 mL | 0.48 mL | 0.48 mL | / | / |
| dd$H_2O$ | 0.70 mL | 1.02 mL | 1.125 mL | 0.926 mL | 1.344 mL |
| 10%APS | 40μL | 40μL | 40μL | 20 μL | 20 μL |
| TEMED | 5μL | 5μL | 5μL | 3μL | 3μL |

**Gel preparation and dyeing precautions:**

1. Prepare separation glue first, then prepare sandwich glue after polymerization, and finally prepare concentrated

glue. The colloidal product ratio of 3 kinds of glue is 4:1.5:1. When electrophoresis, 30v run for 1-2 hours, when the indicating front reaches the upper edge of the separation glue, the voltage is adjusted to 100v, to the end of the electrophoresis, the whole electrophoresis process takes about 6-8 hours.

2. Due to the small number of amino acids contained in the polypeptide, if the polypeptide contains too many polar amino acids (basic or acidic), it will affect its band mobility on the SDS-PAGE chart, that is, its apparent molecular weight may be a certain distance from the amino acid theory of the peptide.

3. Due to the SDS-PAGE map, the molecular weight range of the molecular weight of the protein is proportional to the linear relationship between the logarithmic molecular weight and the mobility is 15,000-200,000, so the molecular weight of the protein or peptide with a molecular weight of less than 10000 can only be estimated according to the standard molecular weight, and whether it falls into the predicted molecular weight range can be inferred.

4. Due to ultra-low molecular weight polypeptides (3000 and below), it is easy to leach from the gel, so the dyeing and decolorization time should not be too long, and the gel should not be soaked in water and preserved for too long after decolorization, otherwise the bands will disappear.

5. After electrophoresis, the glue can be placed in the fixed solution for 20 minutes, and then dyed to get a good protein band; If time does not allow, also can not be fixed directly dyeing. If formula 7 does not work well or if toxicity is considered, please choose our Coomas Bright blue Glue Quick dyeing solution (article No. :P1300), which is a fast, non-toxic and sensitive alternative to conventional dyeing solutions.

**Related Products:**

| | |
|---|---|
| *P1015* | *4× Protein Loading buffer (DTT included)* |
| *P1016* | *4× Protein loading buffer (containing sulfhydryl reducing agent)* |
| *T1070* | *5× TRIS-Glycine electrophoresis buffer* |
| *P1300* | *Coomassie Bright Blue quick stain* |

**Attachment: Preparation of small molecule protein SDS-PAGE electrophoresis reagent**

1. 49.5% T 3% C (Preparation of sandwich glue and concentrated glue)

48g acrylamide

Methyl diacrylamide 1.5g

Dissolve with dd$H_20$ and set volume to 100mL

2. 49.5% T 6% C (Prepare separation glue)

Acrylamide 46.5g

Methylenediacrylamide 3.0g

Dissolve in dd$H_20$ and volume to 100mL

3 Gel buffer

Tris base 182g

dd$H_2O$ 300mL

Adjust pH to 8.45 with HCl

Set the volume to 500mL with $ddH_2O$

Add another 1.5g of SDS

4. 10 x anode buffer (lower tank buffer)

Tris base 121.1 g

$ddH_2O$ 400mL

Adjust pH to 8.9 with HCl

Set volume to 500mL with $ddH_2O$

5. Cathode buffer (upper tank buffer)

Tris base 12.11g

Tricine 17.92g

SDS 1g

Set volume to 1000mL with $ddH_2O$

No pH adjustment is required for this solution

6 Fix the solution

0.5% glutaraldehyde

30% ethanol

Volume to 100mL with $ddH_2O$

7 Dye the solution

50% methanol

10% acetic acid

0.2% Coomassie Bright Blue G-250

Volume up to 500mL with $ddH_2O$