Cat.:BR4C303

180 kDa Prestained Protein Marker

V01

BIORI BIOTECH

Product Description

180 kDa Prestained Protein Marker consists of 10 prestained proteins in three colors with molecular weights ranging from 13-180 kDa. The 75 kDa band is orange-red, the 25 kDa band is green, and the 13 kDa, 17 kDa, 36 kDa, 45 kDa, 60 kDa, 100 kDa, 140 kDa, and 180 kDa bands are blue. This product is intended for SDS-PAGE and clearly indicates electrophoresis progress while providing a useful reference for estimating target-protein molecular weight. In Western blot experiments, it can be used to monitor transfer performance on PVDF or NC membranes and to help assess target-protein detection. This product is suitable for Tris-Glycine gels and Bis-Tris gels in Tris-Glycine, MOPS, and MES buffer systems. All protein bands have been calibrated against unstained protein markers to ensure accurate band sizes. The recommended loading volume is 5 μL per lane.

Components

Components	BR4C303-01 100 rxns	BR4C303-02 500 rxns
180 kDa Prestained Protein Marker	1×0.5 mL	5×0.5 mL

Note: Buffer composition: 62.5 mM Tris-HCl (pH 7.5), 1 mM EDTA, 2% (w/v) SDS, 10 mM DTT, 33% (w/v) glycerol.

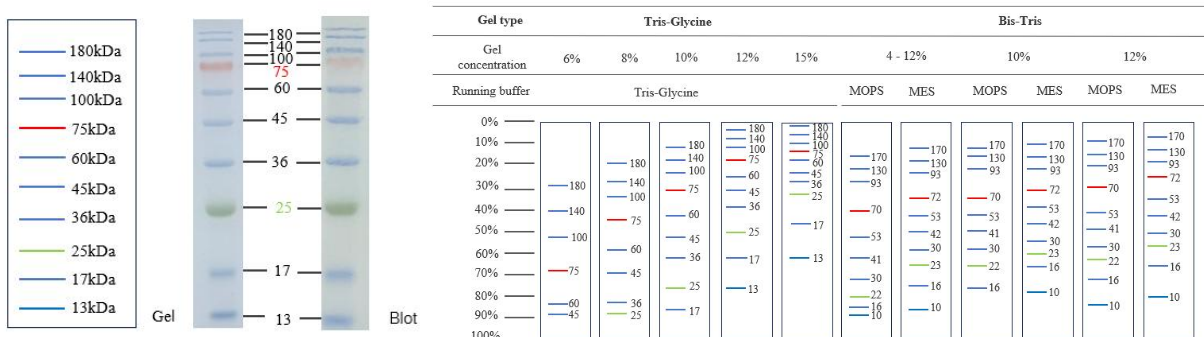
Storage

Store at -20±5°C.

Protocol

1. This product is ready to use. Thaw at room temperature for several minutes, gently mix to ensure homogeneity, and briefly centrifuge before use.
2. Load 5 μL of the sample into each well for gel electrophoresis. If the wells are wider or the gel is thicker, the loading volume may be increased appropriately.

The figure below shows the band-molecular-weight diagram of the prestained protein marker and its migration patterns under different electrophoresis conditions.



Notes

1. For Research Use Only. Not for use in diagnostic procedures.
2. This product is convenient to use and does not require heating, dilution, or the addition of extra reducing agents. Mix well before use and avoid repeated freeze-thaw cycles. This product is intended for denaturing electrophoresis only and is not suitable for native electrophoresis.
3. In Western blot experiments, transfer of high-molecular-weight proteins (>100 kDa) may require a longer transfer time or a higher current.
4. Prestained protein markers have different migration rates in different SDS-PAGE buffer systems and should be used as a reference only when estimating target-protein molecular weight.
5. A 12% resolving gel is recommended. In low-percentage gels, low-molecular-weight proteins migrate too quickly and may overlap with the bromophenol blue tracking front, reducing resolution.
6. For your safety and health, please wear a lab coat and disposable gloves when operating.

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Troubleshooting and FAQs

1. Why are the low-molecular-weight bands faint or the high-molecular-weight bands faint?

Gel casting: If the interface between the resolving gel and the stacking gel is not sufficiently even, this issue may occur during electrophoresis. Thoroughly mix the gel solution during preparation by gentle inversion, pour the gel at a steady rate to minimize bubbles, allow the resolving gel to solidify completely before inserting the comb, and insert the comb vertically and gently to avoid disturbing the gel surface.

Electrophoresis tank sealing: Poor sealing can cause unstable voltage and produce tilted bands. Before electrophoresis, check the lid locks and gasket condition. If buffer leakage is observed, stop the run, refill the buffer, ensure equal liquid levels and proper sealing, and then restart electrophoresis.

Loading volume: Inaccurate loading volume may contribute to this issue. Recalibrate the loading volume and repeat the run.

Edge effect: Avoid loading samples too close to both sides of the gel. Align the pipette tip with the center of the well and load slowly. If bands at the edges are abnormal, the loading volume in the middle wells may be increased appropriately to compensate for edge effects.

2. Why are the protein marker bands diffuse or blurry?

Gel preparation: Confirm that the resolving-gel concentration matches the molecular-weight range of the marker. The gel must be fully polymerized to avoid band diffusion caused by an unstable gel matrix.

Electrophoresis conditions: Use an appropriate voltage. Excessively high voltage generates heat and can impair band resolution, while excessively low voltage slows migration and can also blur bands. Use freshly prepared electrophoresis buffer, because repeatedly used buffer may have reduced ionic strength and altered pH, which can affect band clarity.

Marker handling and loading: Avoid repeated freeze-thaw cycles by aliquoting if necessary. Fully thaw and mix before use. Excessive loading can overcrowd proteins in the gel and impair separation, whereas insufficient loading produces weak band signals. Use the recommended loading volume of approximately 5 μL.

Staining and destaining: If additional staining is performed, such as Coomassie Brilliant Blue staining, control the staining and destaining times appropriately. Insufficient staining or incomplete destaining can produce high background that obscures bands, whereas overstaining or over-destaining can make bands lighter or blurrier.

3. Why are the bands clear after electrophoresis but lighter after transfer?

Under normal conditions, marker bands usually become more vivid after transfer. If the color becomes lighter, the following factors should be considered:

Transfer temperature is too high: Increased thermal motion can cause proteins to detach from the membrane or suffer structural damage.

Small bubbles or gaps are present between the gel and the filter paper: This can make the electric field uneven and lead to discontinuous or incomplete transfer. Proteins in bubble-blocked areas cannot transfer effectively, resulting in locally lighter or missing bands.

Transfer conditions are not suitable: Excessively long transfer may cause proteins to pass through the membrane, whereas insufficient transfer time can leave proteins in the gel. Excessively high transfer voltage can cause overly rapid migration and uneven transfer. In addition, SDS is generally not recommended in the transfer buffer. If SDS must be used, keep the concentration within 0.02-0.04%.

The membrane has not been fully activated: Excessive methanol concentration can cause proteins to pass through the membrane and transfer onto the filter paper, while inadequate activation can reduce protein-binding capacity.

The membrane pore size is not suitable: A 0.22 μm membrane is recommended for proteins smaller than 20 kDa, and a 0.45 μm membrane is recommended for proteins larger than 20 kDa.