Low Molecular Weight (LMW) Protein Marker II

Catalogue number:

MB21401 (300 lanes)
MB21402 (600 lanes)

Description

The Low Molecular Weight Protein Marker II for SDS electrophoresis is a liquid mixture of eight purified proteins for use in molecular weight estimation in the presence of the detergent sodium dodecyl sulphate (SDS). This protein marker contains no oligosaccharides that may cause anomalous migrations and heterogeneous fuzzy bands. The molecular mass of the protein under investigation is determined by comparing its electrophoretic mobility with that of proteins contained in the kit. Three (MB21401) or six (MB21402) 500 µL vials are supplied, each containing a mixture of highly purified protein standards of molecular mass ranging from 11000 to 96000 when used in denaturing polyacrylamide electrophoresis. The mixture is supplied in a loading buffer for direct loading on gels.

Storage conditions

Low Molecular Weight Protein Marker II should be stored at -85°C to -15°C. If you require more than 5 freeze-thaw cycles, we recommend aliquoting the protein marker into smaller volumes and freezing each aliquot at a constant temperature freezer (preferably store in an ultra-freezer).

Components

Protein mixture (enough for 100 lanes) containing the following proteins:

• Xylanase U4, *Clostridium thermocellum*, relative molecular mass (Mr) 96 000

• Albumin bovine serum, relative molecular mass (Mr) 66 000

• Phosphomanose Isomerase, *Cellvibrio mixtus*, relative molecular mass (Mr) 48 000

• Cellulase 5A, *Cellvibrio mixtus*, relative molecular mass (Mr) 40 000

• Cellulase M9, *Clostridium thermocellum*, relative molecular mass (Mr) 32 000

• Family 4 Carbohydrate esterase, *Clostridium thermocellum*, relative molecular mass (Mr) 26 000

• Xyloglucan-binding domain M6, *Clostridium thermocellum*, relative molecular mass (Mr) 18 500

• PDZ domain, metagenome, relative molecular mass (Mr) 11 000

The amount of each protein has been chosen to give bands of equal intensity when stained with BlueSafe (MB15201) following Laemmli-type gel electrophoresis. Intensities may vary when using other staining methods.

Loading buffer

30% (w/v) glycerol, 2% (w/v) SDS, 25 mM NaHepes, pH 7.5, 50 mM DTT, 5 mM EDTA, 0.02% (w/v) NaN_3.

Recommendations for loading

1. Thaw the marker at 37-40 °C for a few minutes. An initial precipitate is normal due to SDS.

2. Mix gently, but thoroughly, to ensure that the solution is homogeneous and clear.

4. Heat this tube at 95 °C for 5 minutes to completely denature proteins.

5. Chill on ice.

6. Make 50 µL aliquots and keep them at -85°C to -15°C while not in use (additional heating is not required; for next uses, simply thaw the aliquots at room temperature and mix until the solution is homogeneous and clear).

7. Load the following volumes of the marker on SDS-PAGE:

- 5 µL per well for mini-gels;
- 10 µL per well for large gels.

Electrophoresis and Detection

Perform electrophoresis according to the instructions supplied with the gel apparatus being used. Stain the gel using the desired method.

Molecular Weight determination

Measure the migration distance of the protein markers and of the protein(s) of interest. Measure the migration distance of the dye marker. Calculate the corresponding Rf values by dividing migration distance of the protein by migration distance of the dye marker. Construct a calibration curve by graphing Rf vs. log molecular weight for the proteins in the Low Molecular Weight Protein Marker II. Determine the molecular weight of the protein(s) of interest from the calibration curve.

Quality control assays

Purity

5 µL of Low Molecular weight (LMW) Protein Marker II is electrophoresed in a 14% Tris-glycine SDS-PAGE to check the intensity and the pattern of bands. It is expected to observe 8 regularly spaced bands as presented in Figure 1 below.

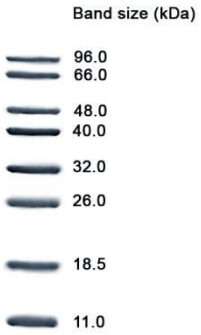

Figure 1. NZYTech LMW Protein Marker II stained with BlueSafe. The gel was loaded with 5 µL of LMW II standard per lane on a 14% Tris-glycine SDS-PAGE.

V2301

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