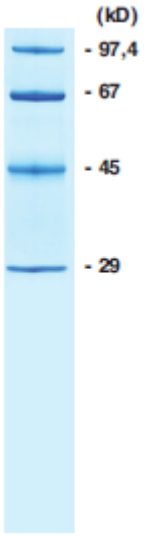
Instructions for use

ROTI®Mark SMALL

Protein-molecular weight marker for SDS-PAGE

not prestained



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I. Introduction

ROTI®Mark SMALL is a protein marker for the small size range. It consists of 4 natural proteins. The concentration of the individual proteins is set in such a way that homogeneous dying intensity can be obtained with Coomassie staining solutions.

The proteins are lyophilised and not pre-treated.

Protein	MW (kDa)
Phosphorylase B	97.4
Albumin bovine (BSA)	67
Ovalbumin	45
Carbonic anhydrase	29

II. Storage

Please store ROTI®Mark SMALL at +4 °C.
The marker is shipped at ambient temperature.

III. Dissolution and Gel loading

- ROTI®Mark SMALL must be dissolved in loading buffer before use. We recommend ROTI®Load 1, 4x conc. (Art. No. K929.1).
- Dissolve 5 mg marker step by step in 5 ml 1x loading buffer (3.75 ml aqua bidest. + 1.25 ml 4x ROTI®Load 1) and transfer to a vial of suitable size. For 10 mg markers, adjust quantities accordingly.

  Final concentration: 1 mg/ml
- Mix solution well with pipette, marker should be completely dissolved.
- Heat solution at 95 °C for 5 mins and aliquot. Freeze unused aliquots at -20 °C.

Before use, carefully heat the aliquot to return precipitated SDS to solution.

Recommended loading amount for mini gels:
(10 x 8 cm; 0.75 or 1 mm thickness)
Coomassie staining: approx. 5 µl
Silver staining: approx. 1 µl

Important: Loading amount required varies depending on gel thickness, C/T ratio, the staining used and width of comb tooth.

The intensity of Coomassie staining can turn out very differently depending on protocol used. Two methods, which guarantee efficient staining, can be found in top V.

IV. Trouble Shooting

Marker-bands cannot/can only be seen very weakly.

- Please ensure the correct loading amount. The recommended quantity is valid for mini gels with a thickness of 0.75 mm. If thicker or larger gels are used, the loading amount must be increased.
- Improve staining. Different staining methods can result in very different results. See V for excellent staining methods. Do not try to compensate weak staining by increasing the protein load. This will result in a change of the running behaviour of the proteins (of your sample as well as of the marker) and in indistinct and thick bands.

- Few weak marker bands: Under certain conditions marker proteins may agglutinate. Resolubilise marker aliquots by incubating for 5 min. at 80 °C. Mix carefully.

Protein-bands/marker-bands are fuzzy.

- Avoid overloading the gel!
- Please ensure that the dissolved marker is not stored at room temperature for a longer period. Place the marker on ice between two gel runs.
- Avoid frequent freezing/thawing of the dissolved marker.
- Long-term storage of the aliquots should always take place at -20 °C.
- Please take care that the gel contains no air bubbles when casting.
- Please ensure that the gel solution is mixed thoroughly when casting.
- Only use high quality acrylamide solutions (e.g. ROTIPHORESE®Gel 30, Art. No.: 3029.1, or Gel 40, Art. No.: 3030.1).
- Avoid overheating the gel. Reduce voltage if required.
- Check the composition and pH-value of the buffer used

Additional bands.

- Marker proteins have been optimised for coomassie staining. In silver staining, weak additional bands may be visible.
- During long storage or multiple freeze- and-thaw cycles, proteins (mainly the trypsin inhibitor) may decompose to a small extent, resulting in an additional very weak band at approx. 10 kDa.

V. Coomassie Staining

With ROTI®Blue (Art. No.: A152.1):

- Incubate gel 2 to 12 h with ROTI®Blue as per instructions.
- Decoloring is not necessary.

With Brilliant Blue G250 (Art. No.: 9598.1):

- Incubate gel for 30-60 minutes in fixative under gentle shaking
- Incubate gel for 20-40 minutes in staining solution under gentle shaking
- Incubate gel for 30 seconds in fixative under gentle shaking
- Incubate gel in decoloring solution under gentle shaking until background staining has been removed and proteins are clearly visible.
- Fixative: 40 % ethanol, 10 % acetic acid
- Staining solution: Mix 50 ml solution I and 50 ml solution II directly before use
  Solution I: 0.2 % Brilliant Blue G250, 90 % ethanol
- Solution II: 20 % acetic acid
- Decolouration solution: 20 % ethanol, 10 % acetic acid

VI. Recommended Reagents

- Brilliant Blue G250, Art. No.: 9598.1
- Ethanol, p.a., Art. No.: 9065.1
- Acetic acid, p.a., Art. No.: 3738.1
- ROTI®Blue, Art. No.: A152.1
- ROTI®Blue quick, Art. No.: 4829.1


Danger H334

ROTI®Mark SMALL	
1LCK.2	**5 mg**
1LCK.3	**10 mg**