Instructions for use

ROTI®Mark PETIT

Protein molecular weight marker for SDS-PAGE

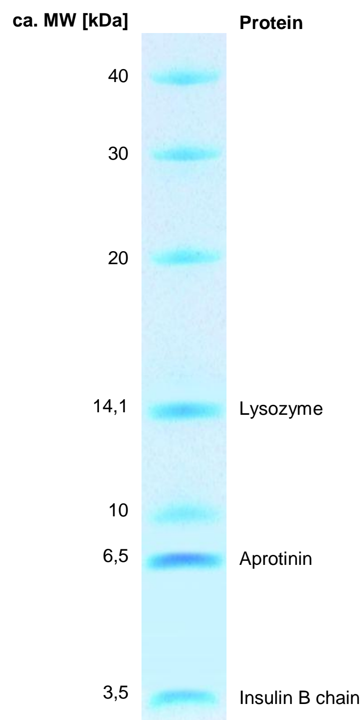

Figure: 5 µl ROTI®Mark PETIT in Tris-Tricine Gel 16,5 %

I. Introduction

ROTI®Mark PETIT is a ready-to-use protein molecular weight marker für polyacrylamide gels. It is composed of three natural proteins (Lysozym, Aprotinin and Insulin B chain) and four recombinant proteins with a molecular weight of 10, 20, 30 and 40 kDa.
The proteins are pre-reduced, acylated and dissolved in non-reducing Laemmli buffer with 8.7 % glycerol and 0.01 % bromphenol blue.

The concentration of the individual proteins ranges between 0.1 and 0.2 mg/ml. It is set in such a way that homogeneous dyeing intensity can be obtained with Coomassie staining solutions.

The marker is *not* suitable for concentration analysis of protein solutions.

ROTI®Mark PETIT is an ideal addition to the protein marker ROTI®Mark 10-150 (Art.-No. T850).

II. Storage

- Please store ROTI®Mark PETIT at –20 °C. The marker can be stored at 4 °C for a short period (a few days). To avoid frequent freezing and thawing, aliquots should be frozen.
- Please let the marker come up to room temperature slowly before use. ROTI®Mark PETIT is pre-reduced and acylated. Therefore, heating prior to use (30-60 sec at 70 °C) is generally not required but can, however, increase band sharpness.
- **The marker should not be stored for a longer period at temperatures above freezing point!**

III. Gel Loading

- Recommended loading amount for mini gels (10 %; 0.75 mm thick):
  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 top 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. (alt. 1 min at 95 °C). Mix carefully.

Protein bands/ marker bands are fuzzy.

- Avoid overloading the gel!
- Please ensure that the 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 marker.
- Long-term storage 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, or Gel 40, Art. No.: 3030).
- 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 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):

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

With Brilliant Blue G250 (Art. No. 9598):

- Incubate gel 30-60 min in fixative under gentle shaking.
- Incubate gel 20-40 min in staining solution under gentle shaking.
- Incubate gel 30 sec in fixative under gentle shaking.
- Incubate gel in decolourising solution under gentle shaking until background staining has been removed and proteins are clearly visible.

Fixative:
40 % ethanol, 10 %

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

Decolourising solution:
20 % ethanol, 10 % acetic acid

VI. Recommended Reagents

Brilliant Blue G250, Art. No.: 9598

Ethanol, p.a., Art. No.: 9065

Acetic acid, p.a., Art. No.: 3738

ROTI®Blue, Art. No.: A152

ROTI®Mark PETIT

9299.1	**100 µl**
9299.2	**500 µl**

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