Instructions for use
ROTI®Mark
WESTERN PLUS

ready-to-use

Prestained protein molecular weight marker for SDS PAGE & detection on Western blots

- Large molecular weight range: 15-200 kD
- 10 IgG-binding and 4 prestained proteins
- Suitable for most Western blots
- Detection *without additional antibodies* possible
- Suitability for all common detection systems
- Applicable for stripping and reprobing

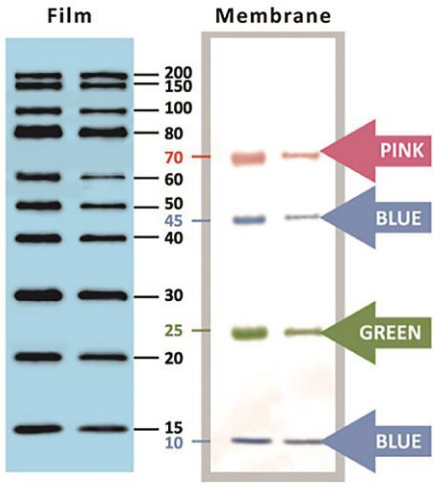

Figure:
ROTI®Mark WESTERN PLUS, unstained,
after detection on X-ray film (left),
after Western blot on PVDF membrane (right)

I. Introduction

ROTI®Mark WESTERN PLUS protein marker covers a wide molecular weight range from 15 to 200 kD.

It consists of ten IgG binding proteins with two in their mass enhanced reference bands (30 kD and 80 kD). For the most common type of primary antibodies are IgG type antibodies the ROTI®Mark WESTERN PLUS marker can be used in almost any situation of Western Blot.

For easier orientation in the gel, as well as for simplified running and transfer control, four additional proteins are pre-colored: 10 kD (blue), 25 kD (green), 45 kD (blue) and 70 kD (pink).

Please note: The prestained proteins of the ROTI®Mark WESTERN PLUS are not suitable for size determination of proteins in the gel.

The molecular weights of the proteins change as a result of being coupled with the dye and vary according to the coupling efficiency. Additionally, the behaviour of the prestained proteins in the gel differs slightly depending on the actual gel and buffer system used. The indicated molecular weight values are, therefore, only approximate. The effective molecular weights may deviate from the values indicated.

II. Composition

Four prestained proteins and ten IgG-binding proteins in aqueous Tris-Glycine buffer solution, mod. acc. to Laemmli (migration patterns in the different buffer systems s. table below).

III. Application

The marker has two functions:

- The four prestained can be used for controlling separation behavior during SDS PAGE and for verification of transfer efficiency during Western blot.
- The ten IgG-binding proteins enable immunodetection on X-ray film or by CCD imaging. They are suitable for all common detection systems like chemiluminescence, fluorescence, color reaction etc.

IV. Storage

- **For a longer period, the marker should be stored at -20 °C!**
- **The marker will not be shipped cooled or with dry ice. This does not affect usability.**
- ROTI®Mark WESTERN PLUS can be stored at +4 °C for up to three months. Nevertheless, we recommend a temperature of -20 °C for long-term storage. To avoid frequent freezing and thawing, aliquots should be frozen.
- Normally, it is not necessary to heat the marker before use. Aggregate bodies may form when used for a longer period or when stored at +4 °C. These can be dissolved if heated for a short period (2-5 min, 60 °C). Do not heat above 80 °C!

V. Gel loading

Recommended loading amount for mini gels up to 1,5 mm thick:

- Two-step Western blot with primary and conjugated secondary antibody: 1,5 – 2,5 µl
- One-step Western blot with only one conjugated antibody: 2,5 – 5 µl

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

VI. Suitable antibodies

- Primary antibodies (IgG)
- Secondary antibodies conjugated with reporter such as HRP, AP, Biotin, Alexa Fluor®, IRDye®

VII. Compatibility

The ROTI®Mark WESTERN PLUS protein marker is compatible with

- All gel solutions and ready-to-use gels available on the market
- PVDF and nitrocellulose membranes
- Semi-dry and tank blotting systems
- Powdered milk and ROTI®Block as a blocking reagent. Please note: Using BSA can lead to an enhanced background
- PBS/PBST and TBS/TBST as buffer systems

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VIII. Different migration patterns

Depending on the buffer system used, the approximate molecular weight of the pre-dyed bands varies:

Band	Color	Tris-Glycine	Bis-Tris (MOPS)	Bis-Tris (MES)
1	Pink	70	61	62
2	Blue	45	41	42
3	Green	25	22	23
4	Blue	10	9	10

IX. Trouble Shooting

Marker bands cannot/can only be seen very weakly:

- The loading amounts specified for marker and antibody dilution are standard values and are to be adapted to the system used. They can vary depending on the blot membrane applied, the transfer buffer, blocking solution, peroxidase-substrate and detection system.
- Few weak marker bands: Under certain conditions marker proteins may agglutinate. Resolubilise marker aliquots by incubating for 2-5 min at 60 °C. Do not heat above 80 °C!
- Marker band signals which are too weak can be caused by bad transfer efficiency during blotting. Please observe the blot times and the manufacturers' instructions.

High background:

- Please ensure thorough washing after antibody incubation.
- Test other blocking solutions. Blocking with 3 % BSA, which is occasionally applied, often leads to a high background. Rather use, for example, 1 % low-fat powdered milk in TBST or in PBST or 1 x ROTI®Block.

X. Further reagents

Buffer reagents:

ROTI®Fair TBST (Art.-No. 1248)
ROTI®Fair PBST (Art.-No. 1115 or 1116)
ROTI®PreMix PBST (Art.-No. 0987)
ROTI®Stock 10x PBST (Art.-No. 1059)
ROTI®Stock 10x TBST (Art.-No. 1061)

Blocking reagents:

Albumin solution 20 % (Art.-No. 9400)
Albumin solution 30 % (Art.-No. 9401)
Albumin Fraction V, pH 5.2 (Art.-No. 2834)
Albumin, IgG free (Art.-No. 3737)
Sodium-Casein (Art.-No. 8569)
Powdered milk (Art.-No. T145)
ROTI®Block, 10x conc. (Art.-No. A151)
ROTI®ImmunoBlock, 10x conc. (Art.-No. T144)

The marker is delivered in gel loading buffer and is immediately ready for use.

No heating, dilution or addition of reducing agents necessary!

ROTI®Mark WESTERN PLUS

2245.1	**250 µl**
2245.2	**500 µl (2 x 250 µl)**