Technical Data Sheet

WesternFroxx pure

for immunodetection

Order number: **1934**

The special 10X wash buffer can be ordered separately: 5570ML500, 1 x 500 ml

WesternFroxx enables **HRP-based immunodetection in one step with one solution only**. Blocking and binding of the primary and secondary antibody occur simultaneously with highest specificity and sensitivity. Blotting procedure, membranes and signal detection do not have to be modified with WesternFroxx. Proteins are detected by ECL or other HRP substrates. Despite the fast incubation process, WesternFroxx leads to clear and distinct bands suitable for publishing. The reason: WesternFroxx solution does block the membrane much faster and more effectively compared to standard blockers. Therefore, the primary and secondary antibodies don't bind to the membrane non-specifically and a faster incubation process is enabled. Furthermore, by using the special Washing buffer after blotting, the membrane is released from components of the transfer buffer (e.g. methanol or ethanol) and perfectly prepared for the extremely fast blocking step.

CAUTION: WesternFroxx is optimized and tested for HRP-coupled secondary antibodies. Alkaline Phosphatase is not stable in WesternFroxx and will lose its activity after a few days. There are no data available regarding Fluorophore-, or Biotin-coupled detection antibodies so far. In case of using these antibodies, we recommend to test a sample first.

Kit components

The kit contains the WesternFroxx detection solution and a special 10X washing buffer.

Solution 1 **10X Washing buffer**

Solution 2 **WesternFroxx pure** containing blocking reagent

Preparation

Before you start with WesternFroxx

- Transfer your proteins to the membrane of choice (PVDF, nitrocellulose) as usual.
- Dilute the provided 10X washing buffer (Solution 1) with water.
- Add your secondary antibody into 20 ml of WesternFroxx pure (Solution 2). The final concentration should be 0.2 to 0.5 µg/ml. Mix gently and incubate at RT for 5 min.
- Add also the primary antibody (0.2 to 0.5 µg/ml) to the WesternFroxx Solution 2.

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Protocol

1. Wash the membrane 3 x 5 min. while shaking with 20 ml of 1X Solution 1 (minigel).
2. Incubate the membrane with WesternFroxx Solution 2 (containing primary and secondary antibody) for 15 min. to 1 hour. **Caution!** The WesternFroxx solution can be reused up to 5 times. Kept in the fridge it is stable for up to 6 months.
3. Wash the membrane again for 3 x 5 min with 1X Solution 1.
4. Start the detection with ECL-Solution (we recommend ECL Xtrasensitivity Kit, order number 5560).

Related products

WesternFroxx is also available as anti-rabbit HRP (order number 6666) and anti-mouse HRP (order number 5555) variant, containing already a secondary detection antibody.

6666	WesternFroxx Kit anti-rabbit HRP for immunodetection
5555	WesternFroxx Kit anti-mouse HRP for immunodetection
7777	WesternFroxx all-in-one Protein Ladder (15 – 200 kDa) for immunodetection
1052	Protein Ladder (6.5 - 212 kDa), unstained for molecular biology
1123	Protein Ladder (11 – 245 kDa), prestained for molecular biology
1288	Protein Ladder (10 – 180 kDa), prestained for molecular biology
5560	ECL Xtrasensitivity Kit for immunodetection
1550	BSA blocking solution for immunodetection
1080	CleanBlot Background Minimizer for immunodetection
1095	Peptide blocking solution (BSA-free) for immunodetection
1080	Casein high-end blocking solution for immunodetection
1102	Acrylamide Xtra solution 30 % - Mix 37.5:1 for electrophoresis
1248	Acrylamide Xtra solution 40 % - Mix 37.5:1 for electrophoresis
1106	Acrylamide Xtra solution 30 % - Mix 29:1 for electrophoresis
1912	Coomassie® brilliant blue R-250 (C.I. 42660) for biochemistry
1277	SDS Xtrapure for biochemistry
8027	TEMED for biochemistry
1610	Ammonium persulfate for molecular biology
1125	Tris Xtrapure for biochemistry
1275	Glycine for biochemistry

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