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Western Blocking Reagent

10 × conc. Blocking solution for Western blots

Cat. No. 11 921 673 001 100 ml (approx. 10 blots à 100 cm^2)
Cat. No. 11 921 681 001 6 × 100 ml (approx. 60 blots à 100 cm^2)

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1. What this Product Does

Formulation

The blocking solution contains 10% of purified casein protein in maleic acid buffer.

Stability

The blocking reagent is unopened stable at +2 to +8°C until the expiry date given on the package. After opening the content of the bottle is stable for 4 weeks if stored at +2 to +8°C.
For longer periods it is recommended to store the content of the bottle in aliquots at −15 to −25° C.

Working Procedure for standard Western Blots

The procedure is described for a standard Western blot with colorimetric or chemiluminescent detection.

2. Preparation of Working Solutions

Tris Buffered Saline (TBS), pH 7.5

To 800 ml redist. water add 6.05 g Tris base (50 mM), 8.76 g sodium chloride (150 mM) and adjust the pH to 7.5 with approx. 9.5 ml 1 M hydrochloric acid.
Then dilute to 1 l with double dist. water. TBS is stable for 3 months, when stored at +2 to +8°C.

Since sodium azide inhibits POD, it must not be used as antimicrobial agent when using POD-conjugates.

TBS-Tween (TBST)

Washing buffer 1: Dissolve 1 ml Tween 20* in 1 l of TBS. TBST is stable for 3 months, when stored at +2 to +8°C.

0.1% Tween 20 is suitable for the most applications, but – depending on the membrane and on the antibody used – different detergents (like SDS, Triton X-100* and Nonidet P-40*) and detergent concentrations from 0.01–1% may lead to better results.

Blocking Solution (1%)

Dilute 10 ml Western blocking reagent (10 × conc.) in 90 ml TBS. The Blocking solution can be stored at +2 to +8°C for 1 month.

Since sodium azide inhibits POD, it must not be used as antimicrobial agent when using POD-conjugates.

Blocking Solution (0.5%)

Dilute 50 ml Blocking solution (1%) with 50 ml of TBS.

Antibody Solutions

Dilution and incubation solution for all antibodies is 0.5% Blocking reagent in TBS (see above). In order to exploit the full detection potential of the system we recommend to optimize the dilutions of the primary and secondary antibody in dot blot assays in advance. (Start first with 3 to 4 dilutions of primary antibody and a constant concentration of the second antibody. Then choose the most suitable dilution of primary antibody and optimize the concentration of the secondary antibody in the same way.)

The concentration of the blocking reagent is a powerful tool to improve the signal to noise ratio in Western blots. If high background appears even under optimized antibody concentrations, increase the concentration of the blocking reagent during the antibody incubations and washing steps from 0.5% to 1%.

In case of weak signals even with prolonged antibody incubations lower the concentration of blocking reagent during the antibody incubations and washing steps from 0.5% to 0.1%.

2.1 Immunodetection

Before You Begin

- The membrane must not get dry at any step. If drying occurs rewet PVDF membranes in 5% Tween 20. This, however, may influence antibody binding.
- All steps for immunodetection are performed at +15 to +25° C with gentle agitation on a reciprocal shaker or using a roller incubator.
- For reproducible results equilibrate all solutions to +15 to +25° C before use.
- Do not scratch the membrane, do always use blunt-ended forceps with non serrated tips.

Membrane Blocking

If blotting was performed in a buffer system containing methanol, briefly wash the membrane with TBS.

Block unspecific binding of antibody by incubating the membrane for 1 h in 1% blocking solution. Alternatively this step can be performed overnight at +2 to +8°C without shaking.

Primary Antibody

Incubate membrane for 1 h with primary antibody diluted in 0.5% blocking solution. This step can also be performed at +2 to +8° C overnight without shaking. Incubation time may be longer if either the affinity of the antibody to the antigen, or the concentration of specific antigen is low. The optimum concentration of primary antibody should be evaluated as detailed in the previous section (I.).

Washing

Wash twice in TBST for 10 min each, then wash twice with 0.5% Blocking solution for 10 min each. For efficient washing always use large volumes of washing buffer (approx. 50 ml for a 10 × 10 cm membrane).

Secondary Antibody

Incubate membrane for 1 h with secondary antibody diluted in 0.5% Blocking solution. The optimum concentration of secondary antibody should be evaluated as detailed in the previous section (I.).

The following guiding values for the working concentrations are valid for the antibody conjugates from Roche Diagnostics.

Conjugate working	Working concentration for colorimetric detection	Working concentration for chemiluminescent detection
Anti-mouse-Ig-POD, Fab fragments	300 mU/ml	50 mU/ml with Lumi-Light^{PLUS} Western Blotting Substrate; 100 mU/ml with Lumi-Light Western Blotting Substrate
Anti-rabbit-IgG-POD	750 mU/ml	20 mU/ml with Lumi-Light^{PLUS} Western Blotting Substrate; 100 mU/ml with Lumi-Light Western Blotting Substrate
Anti-mouse-Ig-AP, Fab fragments	800 mU/ml	80 mU/ml CDP-Star
Anti-rabbit-IgG-AP	800 mU/ml	80 mU/ml CDP-Star

Washing

Wash 4 × in TBST for 15 min each with large volumes of TBST.

2.2 Detection

The procedures for AP and POD substrates, as well as for colorimetric and chemiluminescent detection differ considerably. Therefore, we refer to the detailed working procedures, practical hints and trouble shooting as described in the respective pack inserts of the kits and single reagents listed below.

Colorimetric detection

- BM Blue POD-substrate, precipitating (TMB solution, ready-to-use)
- BM Purple AP-substrate, precipitating (NBT/BCIP solution, ready-to-use)
- NBT/BCIP ready-to-use tablets
- NBT/BCIP stock solution

Detection using chemiluminescence

- BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)
- BM Chemiluminescence Blotting Substrate (POD)
- BM Chemiluminescence Blotting Kit (Biotin/Strepavidin)
- Lumi-Light Western Blotting Substrate
- Lumi-Light^{PLUS} Western Blotting Substrate

3. Ordering Information

Product	Pack size	Cat. No.
BM Blue POD-substrate, precipitating (TMB solution, ready-to-use)	100 ml	11 442 066 001
BM Purple AP-substrate, precipitating (BCIP/NBT solution, ready-to-use)	100 ml	11 442 074 001
NBT/BCIP ready to use tablets	20 tablets	11 697 471 001
NBT/BCIP stock solution	8 ml	11 681 451 001
BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)	for 2000 cm^2 of membrane	11 520 709 001
BM Chemiluminescence Blotting Substrate (POD)	for 1000 cm^2 of membrane for 4000 cm^2 of membrane	11 500 708 001 11 500 694 001
PVDF Western Blotting Membranes	1 roll 30 cm × 3 m	03 010 040 001
Lumi-Light Western Blotting Substrate	400 ml (4000 cm^2 membrane)	12 015 200 001
Lumi-Light^{PLUS} Western Blotting Substrate	100 ml (1000 cm^2 membrane)	12 012 196 001

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Changes to Previous Version

Editorial Changes

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