For life science research only.
Not for use in diagnostic procedures.

Western Blocking Reagent

Version: 08
Content Version: April 2024

10x-concentrated blocking solution for western blots.

Cat. No. 11 921 673 001	100 mL 10 blots, 100 cm^2
Cat. No. 11 921 681 001	6 x 100 mL 60 blots, 100 cm^2

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Western Blocking Reagent, 10x conc.	Blocking solution containing 10% of purified casein protein in maleic acid buffer.	11 921 673 001	1 bottle, 100 mL
			11 921 681 001	6 bottles, 100 mL each

1.2. Storage and Stability

Storage Conditions (Product)

When stored unopened at +2 to +8°C, the product is stable through the expiration date printed on the label.

Vial / bottle	Label	Storage
1	Western Blocking Reagent, 10x conc.	Once opened, store at +2 to +8°C for up to 4 weeks. For long-term storage, aliquot and store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Powder-free gloves
- Reciprocal shaker or roller incubator
- Blunt-ended forceps with non serrated tips

For preparation of solutions

See section, **Working Solution** *for information on preparing solutions.*

- TBS (Tris buffered saline)
- TBST (TBS-Tween 20)
- Tris base*
- NaCl
- HCl
- Tween 20*
- Double-distilled water

For immunodetection

- PVDF Western Blotting Membranes*
- Primary antibody, antigen specific
- Secondary antibody conjugate

For colorimetric detection

- BM Purple (AP-substrate, precipitating) (NBT/BCIP solution, ready-to-use)*
- NBT/BCIP stock solution*

For chemiluminescence detection

- BM Chemiluminescence Blotting Substrate (POD)*
- Lumi-Light^{PLUS} Western Blotting Substrate*

1.4. Application

The Western Blocking Reagent is used for a standard western blot with colorimetric or chemiluminescent detection onto PVDF membranes.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Membrane handling requirements

Follow good laboratory practice when handling membranes.

- Do not scratch the membrane. Handle membrane only on the edges and with clean blunt-ended forceps with non serrated tips.
- Clean scissors with an ethanol moistened towel before cutting the membrane.
- Wear powder-free gloves to avoid damage or contamination.
- Make sure that there is sufficient solution to entirely cover the membrane.

Special handling of PVDF membranes

PVDF membranes must not dry out at any step. If drying occurs, re-wet membranes in 5% Tween 20 (v/v). This may however influence antibody binding.

Membrane blocking

- If blotting was performed in a buffer system containing methanol, briefly wash the membrane with TBS.
- Block nonspecific binding of antibody by incubating the membrane for 1 hour in 1% Blocking solution.

  Alternatively, this step can be performed overnight at +2 to +8°C without shaking.

Primary antibody

- Incubate membrane for 1 hour 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 section, **Working Solution**.

Washing

- For efficient washing, always use large volumes of washing buffer, approximately 50 mL for a 10 × 10 cm membrane.
- Wash twice in TBST for 10 minutes each, then wash twice with 0.5% Blocking solution for 10 minutes each.

Secondary antibody

- Incubate membrane for 1 hour with secondary antibody diluted in 0.5% Blocking solution.
- The optimum concentration of secondary antibody should be evaluated as detailed in section, **Working Solution**.
- The following working concentrations are starting guidelines for the given secondary antibody conjugates:

Conjugate	Colorimetric detection Working conc. [mU/mL]	Chemiluminescent detection with selected substrate Working conc. [mU/mL]
Anti-mouse-Ig-POD, Fab fragment	300	50 (Lumi-Light^{PLUS} Western Blotting Substrate)
Anti-rabbit-IgG-POD	750	20 (Lumi-Light^{PLUS} Western Blotting Substrate)
Anti-mouse-Ig-AP, Fab fragments	800	80 (CDP-*Star*)
Anti-rabbit-IgG-AP	800	80 (CDP-*Star*)

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

Safety Information

Precautions

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material can vary, the operator must optimize pathogen inactivation and follow the appropriate measures according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online at documentation.roche.com, or upon request from the local Roche office.

Working Solution

Preparation of working solutions		
Solution	**Preparation**	**Storage and stability**
Tris buffered saline (TBS), pH 7.5 (50 mM Tris, 150 mM NaCl)	▪ Dissolve 6.05 g Tris base* and 8.76 g NaCl in 800 mL double-distilled water. ▪ Adjust pH to 7.5 with approximately 9.5 mL 1 M HCl. ▪ Dilute up to 1,000 mL total volume with double-distilled water. ⚠ ***Do not use sodium azide as an antimicrobial agent as it inhibits POD.***	Store at +2 to +8°C for up to 3 months.
TBS-Tween (TBST)	Dilute 1 mL Tween 20* to 0.1% (v/v) final concentration in 1 L TBS. ⓘ *0.1% Tween 20 is suitable for the most applications, but depending on the membrane and on the antibody used, different detergents, such as SDS, Triton X-100, and Nonidet P-40, and detergent concentrations from 0.01 to 1% may lead to better results.*	Store at +2 to +8°C for up to 3 months.
Blocking solution (1%)	Dilute 10 mL Western Blocking Reagent, (10x conc.) in 90 mL TBS. ⚠ ***Do not use sodium azide as an antimicrobial agent as it inhibits POD.***	Store at +2 to +8°C for up to 1 month.
Blocking solution (0.5%)	Dilute 50 mL Blocking solution (1%) with 50 mL of TBS.	Store at +2 to +8°C for up to 1 month.
Antibody solutions	Dilution and incubation solution for all antibodies is Blocking solution (0.5%) in TBS. In order to exploit the full detection potential of the system, 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 secondary antibody. ▪ 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 an important parameter for improvement of 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%.* ▪ For 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%.	⚠ ***Always prepare fresh.***

2.2. Protocols

Standard immunodetection

Here are general guidelines for a standard western blot with colorimetric or chemiluminescent detection.
The procedures for AP and POD substrates, as well as for colorimetric and chemiluminescent detection differ considerably. Therefore, see the detailed working procedures, practical hints, and troubleshooting as described in the respective Instructions for Use of the kits and single reagents listed below.

- 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.

Colorimetric detection

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

Chemiluminescence detection

- BM Chemiluminescence Blotting Substrate (POD)
- Lumi-Light^{PLUS} Western Blotting Substrate

3. Supplementary Information

3.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

3.2. Changes to previous version

Layout changes.
Editorial changes.

3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
NBT/BCIP Stock Solution	8 mL	11 681 451 001
BM Chemiluminescence Western Blotting Substrate (POD)	1 set, 1,000 cm^2 membrane (trays), 6,250 cm^2 membrane (transparent plastic bags)	11 500 708 001
	1 set, 4,000 cm^2 membrane (trays), 25,000 cm^2 membrane (transparent plastic bags)	11 500 694 001
BM Purple	100 ml	11 442 074 001
Lumi-Light^{PLUS} Western Blotting Substrate	1 kit, 100 ml (2 x 50 ml) 10 or 100 blots with 10 cm x 10 cm	12 015 196 001
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Tris base	1 kg, *Not available in US*	10 708 976 001
	1 kg	03 118 142 001
	5 kg	11 814 273 001

3.4. Trademarks

All product names and trademarks are the property of their respective owners.

3.5. License Disclaimer

For patent license limitations for individual products please refer to:
Product Disclaimers.

3.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

3.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

3.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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