



EveryBlot Blocking Buffer Instructions



Introduction

EveryBlot Blocking Buffer is an optimized buffer for blocking membranes prior to immunodetection and for antibody dilution during western blotting. This blocker provides a 5-minute blocking time while maintaining maximum sensitivity for all western blots regardless of detection method. Shelf life is 2 years from the date of manufacture at 4°C.

- Reduces nonspecific antibody binding to reduce background while maintaining excellent sensitivity
- For both chemiluminescent and fluorescent detection
- Contains no phosphate-based buffers
- Ideal for use with phospho-specific antibodies
- Complete blocking in 5 min

Instructions for Use

Use EveryBlot Blocking Buffer during western blotting after proteins have been transferred to a membrane. Use it for blocking the membrane and as an antibody diluent during immunodetection.

EveryBlot Blocking Buffer comes ready to use, with no need for resuspension or dilution. There is no need for addition of detergents or other reagents except for fluorescent detection on polyvinylidene difluoride (PVDF), where we recommend the addition of sodium dodecyl sulfate (SDS) to 0.02% in the secondary antibody solution.

For mini-sized blots, use at least 10 ml for blocking and antibody incubation steps. For midi-sized blots, use at least 20 ml. Ensure that there is sufficient buffer to completely cover the membrane during blocking and incubation.

- Block:** Add EveryBlot Blocking Buffer to the membrane and incubate for 5 min with agitation.
- Incubate in primary antibody:** Dilute the primary antibody in full-strength block and incubate the membrane for 1 hr at room temperature with agitation.
Note: Some primary antibodies yield better results with overnight incubation at 4°C.
- Wash:** Wash membrane 5 x 5 min with TBST (Tris buffered saline + 0.05% Tween 20) with agitation.
- Incubate in secondary antibody:** Dilute the secondary antibodies in full-strength block and incubate the membrane for 1 hr at room temperature with agitation.
Note: For fluorescent detection on PVDF, add SDS to 0.02% in the secondary antibody solution.
- Wash:** Wash membrane 6 x 5 min with TBST with agitation.
- Image:** Proceed to fluorescent imaging. For chemiluminescent and colorimetric detection, apply substrate and image.

Ordering Information

Catalog #	Description
12010020	EveryBlot Blocking Buffer, 500 ml
12010947	EveryBlot Blocking Buffer, 50 ml

Related Products

Catalog #	Description
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StarBright Blue Secondary Antibodies

12005866	StarBright Blue 520 Goat Anti-Mouse IgG, 400 µl
12005867	StarBright Blue 520 Goat Anti-Mouse IgG, 80 µl
12005869	StarBright Blue 520 Goat Anti-Rabbit IgG, 400 µl
12005870	StarBright Blue 520 Goat Anti-Rabbit IgG, 80 µl
12004158	StarBright Blue 700 Goat Anti-Mouse IgG, 400 µl
12004159	StarBright Blue 700 Goat Anti-Mouse IgG, 80 µl
12004161	StarBright Blue 700 Goat Anti-Rabbit IgG, 400 µl
12004162	StarBright Blue 700 Goat Anti-Rabbit IgG, 80 µl

hFAB Rhodamine Housekeeping Protein Fluorescent Primary Antibodies

12004163	hFAB Rhodamine Anti-Actin Primary Antibody, 200 µl
12004164	hFAB Rhodamine Anti-Actin Primary Antibody, 40 µl
12004165	hFAB Rhodamine Anti-Tubulin Primary Antibody, 200 µl
12004166	hFAB Rhodamine Anti-Tubulin Primary Antibody, 40 µl
12004167	hFAB Rhodamine Anti-GAPDH Primary Antibody, 200 µl
12004168	hFAB Rhodamine Anti-GAPDH Primary Antibody, 40 µl

Chemiluminescent Substrates

1705060	Clarity Western ECL Substrate, 200 ml
1705061	Clarity Western ECL Substrate, 500 ml
1705062	Clarity Max Western ECL Substrate, 100 ml

Trans-Blot Turbo Transfer System

1704150	Trans-Blot Turbo Transfer System
1704156	Trans-Blot Turbo Mini PVDF Transfer Packs
1704157	Trans-Blot Turbo Midi PVDF Transfer Packs
1704158	Trans-Blot Turbo Mini Nitrocellulose Transfer Packs
1704159	Trans-Blot Turbo Midi Nitrocellulose Transfer Packs

For technical support, call your local Bio-Rad office. In the U.S., call **1-800-4BIORAD (1-800-424-6723)**.

Visit bio-rad.com/EveryBlot for more information.

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Clarity Max Western ECL Substrate is manufactured by Cyanagen Srl and is the subject of patent application numbers US7855287, EP1950207, US9040252, AU2011202658, CA2742025, US8129136, and EP1962095, together with other equivalent granted patents and patent applications in other countries like CN102313732.

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