INSTRUCTIONS



PierceTM Clear Milk Blocking Buffer (10X)

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37587

Number

Description

37587 Pierce Clear Milk Blocking Buffer (10X), 100mL

Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce Clear Milk Blocking Buffer (10X) is a proprietary pre-formulated milk solution for blocking excess nonspecific binding sites, reducing background in Western blotting applications and diluting antibodies when used with nitrocellulose and PVDF membranes. Pierce Clear Milk Blocking Buffer (10X) provides lower background, enhanced sensitivity, extended shelf life and reproducible results.

Important Product Information

- Milk protein-based products may contain biotin, which interferes with streptavidin and avidin systems. When using streptavidin and avidin enzyme conjugates, use Thermo Scientific Blocker BSA (Product No. 37520 and 37525), SuperBlock Blocking Buffer (Product No. 37515 and 37535) or StartingBlock Blocking Buffer (Product No. 37538 and 37542), which do not contain endogenous biotin.
- Because no blocking reagent is optimal for all systems, empirical testing is essential to determine the appropriate blocking buffer for each system. Determining the proper blocking buffer can increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent.
- For initial testing, use Pierce Clear Milk Blocking Buffer (10X) at a 1X concentration after diluting in ultrapure water. However, other concentrations may be beneficial for specific systems.
- For optimal results, use phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05% TweenTM-20 Detergent (Product No. 28320) **OR** Thermo Scientific Pierce Fast Wash Buffer 10X (Product No. 37577) as wash buffers.

Note: Before use, Pierce Fast Wash Buffer (10X) should be diluted to 1X in ultrapure water.

Procedure for Blocking Nonspecific Binding Sites

- 1. After the transfer, wash membrane briefly in ultrapure water.
- 2. Dilute Pierce Clear Milk Blocking Buffer (10X) to 1X in ultrapure water.
- 3. Add 10-20mL to the membrane and block for 30-60 minutes at room temperature with slight agitation.
- 4. Prepare primary antibody in 1X Clear Milk Blocking Buffer and incubate the blotting membrane for 60 minutes at room temperature (or overnight at 4°C) with slight agitation.

Note: Evaluate each specific antibody/antigen to determine compatibility with the incubation time.

- 5. Wash the membranes for 10 minutes with the wash buffer. Repeat for a total of three washes.
- 6. Prepare secondary antibody in PBS containing 0.05% Tween-20 Detergent **OR** TBS containing 0.05% Tween-20 Detergent and incubate the membrane for 60 minutes at room temperature with slight agitation.

Note: For best results, optimize the secondary antibody concentration and incubation time.

Wash the blotting membrane for 5 minutes with wash buffer. Repeat for a total of four washes. Wash the blotting membrane for 5 minutes with TBS.



- 8. Place the membrane in a clean tray containing the substrate of choice (chemiluminescent or colorimetric).
- 9. Expose to X-ray film or image with a CCD camera.

Additional Information on Our Website

- Tech Tip #24: Optimize antigen and antibody concentrations for Western blots
- Tech Tip #32: Guide to enzyme substrates for Western blotting
- Tech Tip #33: Guide to enzyme substrates for ELISA
- Tech Tip #16: Block endogenous biotin

Related Thermo Scientific Products

XP04200BOX	Novex TM Tris-Glycine protein gels (see <u>thermofisher.com/proteingels</u> for a complete listing)
NW04120BOX	Bolt™ Bis-Tris Plus protein gels (see <u>thermofisher.com/proteingels</u> for a complete listing)
34580	SuperSignal TM West Pico Plus Chemiluminescent Substrate
34075	SuperSignal West Dura Extended Duration Substrate, 100mL
21059	Restore TM Western Blot Stripping Buffer, 500mL
21065	Pierce Background Eliminator Kit
34090	CL-XPosure TM Film, 5" × 7" sheets, 100 sheets/pkg
28376	BupH TM Tris Buffered Saline Packs, 40 packs
28372	BupH Phosphate Buffered Saline Packs, 40 packs
37527	SEA BLOCK™ Blocking Buffer, 500mL
37528	Blocker Casein in PBS, 1L
37520	Blocker BSA in TBS (10X), 125mL

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This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at thermofisher.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

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