INSTRUCTIONS

Pierce™ Fast Blocker

37575 37576

2399.0

Number	Description
37575	**Pierce Fast Blocker,** 100mL, contains a proprietary protein formulation in Tris-buffered saline at pH 7.5
37576	**Pierce Fast Blocker,** 500mL, contains a proprietary protein formulation in Tris-buffered saline at pH 7.5

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

Introduction

The Thermo Scientific Pierce Fast Blocker is a blocking buffer for optimizing Western blotting systems with background signal issues. Pierce Fast Blocker offers compatibility with antibodies and biotin/avidin systems and a short blocking time of 5 minutes, which saves time while also yielding results comparable to classic Western blotting blockers.

Important Product Information

- Use Pierce Fast Blocker at the supplied concentration; do not dilute.
- Pierce Fast Blocker is a saturated solution and settling may occur. Shake the bottle before use. For best results, use a shaking platform during incubation steps.
- Use Pierce Fast Blocker with blots that are not pre-blocked. Pre-blocking the membrane can result in decreased assay sensitivity.
- Do not handle membranes with ungloved hands. Wear gloves or use clean forceps to handle blots.
- For optimal results, use an appropriate wash buffer [e.g., phosphate-buffered saline containing 0.05% Tween™-20 Detergent (Product No. 28320) (PBS-T) or Tris-buffered saline containing 0.05% Tween-20 Detergent (Product No. 28320) (TBS-T) **OR** Thermo Scientific Pierce Fast Wash Buffer (10X) (Product No. 37577) (see Material Preparation Section)].

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

Material Preparation

Pierce Fast Blocker

Dilute the primary antibody in Pierce Fast Blocker to a concentration from 0.2-10µg/mL. The stability of diluted primary antibody varies depending on the antibody. For best results, prepare dilution immediately before use.

Example: To prepare 1µg/mL from an antibody starting concentration of 1mg/mL, mix 10µL of antibody with 10mL of Pierce Fast Blocker.

Note: Shake Pierce Fast Blocker before use. If needed, add a final concentration of 0.05% Tween-20 Detergent to Pierce Fast Blocker.

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Procedure for Blocking Membranes for Western Blotting

1. Remove the membrane from transfer apparatus and place in a clean incubation tray.
2. Briefly wash blot in a wash buffer to remove the transfer buffer.
3. Add a sufficient volume of Pierce Fast Blocker to cover the membrane and incubate for 5 minutes with shaking.

   Note: If Pierce Fast Blocker is not used as the primary antibody diluent, increase the blocking time to 30 minutes. Evaluate each specific antibody/antigen to determine compatibility with the blocking time.
4. Add the primary antibody prepared in Pierce Fast Blocker or buffer (PBS-T or TBS-T) and incubate with shaking for 60 minutes.

   Note: Evaluate each specific antibody/antigen to determine compatibility with incubation time.
5. Continue the Western blotting procedure.

Troubleshooting

Problem	Possible Cause	Solution
High background	High concentration of secondary antibody	Decrease concentration of the secondary antibody
	Insufficient washing	Use a minimum of 20mL of 1X Fast Wash Buffer for each wash
		Add additional wash cycle after the secondary antibody
	Omitted the brief pre-wash after the transfer to remove the transfer buffer	Wash membrane in 1X Pierce Wash Buffer briefly before starting the Western blotting protocol
Weak signal	Used insufficient quantities of antigen or primary antibody	Strip and re-probe using a higher concentration of primary antibody
		Load higher amount of sample onto the gel
	Inefficient protein transfer	Optimize transfer conditions
Spots within the protein bands	Unevenly hydrated membrane	Hydrate membrane before the transfer according to the manufacturer's instructions
	Bubble between X-ray film and membrane	Remove all bubbles before exposing blot to film

Additional Information from Our Website

- Tech Tip #24: Optimize antigen and antibody concentrations for Western blots
- Tech Tip #67: Chemiluminescent Western blotting technical guide and protocols
- Protein Methods Library: Blocking Buffers for Western Blotting and ELISA

Related Thermo Scientific Products

34080	**SuperSignal™ West Pico Chemiluminescent Substrate,** 500mL
34075	**SuperSignal West Dura Extended Duration Substrate,** 100mL
34095	**SuperSignal West Femto Maximum Sensitivity Substrate,** 100mL
46430	**Restore™ Plus Western Blot Stripping Buffer,** 500mL
21059	**Restore Western Blot Stripping Buffer,** 500mL
34090	**CL-XPosure™ Film,** 5" × 7" sheets, 100 sheets/pkg
21065	**Pierce Background Eliminator Kit,** for eliminating background from X-ray film
37527	**SEA BLOCK Blocking Buffer,** 500mL
37528	**Blocker Casein in PBS,** 1L
37530	**Blocker BLOTTO in TBS,** 1L
37535	**SuperBlock™ Blocking Buffer in TBS,** 1L
28376	**BupH™ Tris Buffered Saline Packs,** 40 packs
28372	**BupH Phosphate Buffered Saline Packs,** 40 packs
25200-44	**Precise™ Protein Gels**
25245-74	**Precise Tris-Glycine Gels**

General Reference

Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Springs Harbor Laboratory, 1998.

Tween is a trademark of Croda International Plc.

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").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

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 *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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