

Convert to SuperSignal[®] West Pico Substrate from ECL Substrate

TR0021.2

Introduction

Thermo Scientific SuperSignal[®] West Pico Substrate and GE Healthcare ECL Substrate are enhanced chemiluminescent, luminol-based, Western blotting substrates for horseradish peroxidase (HRP). Although similar in some respects, the two competing substrate products are not identical, and Western blot procedures that have been optimized for detection with one substrate will not necessarily be directly transferable to the other. SuperSignal West Pico Chemiluminescent Substrate is more sensitive than ECL Substrate, requires less (i.e., more dilute) primary and secondary antibody, and results in a longer-duration signal. The following table describes general Western blotting conditions necessary to successfully convert from standard detection with ECL Substrate Western blot to detection with SuperSignal West Pico Chemiluminescent Substrate.

Comparison of Western Blot Conditions

The following protocol is based on a standard Western blotting system involving an unlabeled primary antibody and an HRP-labeled secondary antibody. Conditions given for ECL Substrate are those suggested by the manufacturer. Antibody dilutions are relative to a 1 mg/ml stock. Wash buffer is Tris-buffered saline containing 0.05% Tween[®]-20 (TBS-T).

Step in Western blot procedure	Conditions when using GE Healthcare ECL Substrate	Conditions when using Thermo Scientific SuperSignal West Pico Substrate
Electrophorese protein samples and transfer them to nitrocellulose membrane according to the membrane manufacturer's recommendation.	Use Hybond-ECL nitrocellulose membrane.	Use any nitrocellulose membrane.
Add blocking buffer and incubate for 1 hour at room temperature (RT).	Use 5% blocking reagent. After blocking, wash membrane 1 × 15 minutes, then 2 × 5 minutes.	Use any appropriate blocking buffer (see Additional Information Section). No wash is required.
Add primary antibody and incubate for 1 hour at RT.	Use 1/100-1/5,000 dilution.	Use 1/500-1/5,000 dilution. (Try 1/1,000 dilution first.)
Wash blot with adequate volumes of wash buffer.	Wash 1 × 15 minutes. Wash 2 × 5 minutes.	Wash 6 × 5 minutes.
Add HRP-labeled secondary antibody and incubate for 1 hour at RT.	Use 1/1,000-1/15,000 dilution.	Use 1/20,000-1/100,000 dilution. (see Additional Information Section).
Wash membrane thoroughly with adequate volumes of wash buffer.	Wash 1 × 15 minutes. Wash 4 × 5 minutes.	Wash 6 × 5 minutes.
Prepare chemiluminescent substrate working solution.	Mix equal volumes of Detection Reagents 1 and 2. The working solution must be used promptly.	Mix equal volumes of the Luminol/Enhancer Solution and Stable Peroxide Solution. The working solution is stable for 8 hours.
Add substrate working solution to cover membrane.	Incubate for precisely 1 minute at RT.	Incubate for ~5 minutes at RT.
Drain away excess substrate working solution, cover membrane with clear plastic, and expose to X-ray film.	Expose membrane to film for 15 seconds. If necessary, make additional exposures for 1 minute to 1 hour. Longer exposures may be required as time passes.	Expose membrane to film for 1 minute. If necessary, make additional exposures for 30 seconds to 5 minutes. Detectable signal will last up to 6 hours.

Additional Information

A. Blocking Buffers

Western blotting involves a unique combination of protein sample and specific antibodies. Without prior knowledge, there is no way to be certain that antibodies will not adversely interact with particular proteins in the blocking buffer. Accordingly, different blocking buffers may have to be tested for compatibility (i.e., low background) with the specific blotting system. Pierce offers a complete line of blocking buffers for Western blotting, including Thermo Scientific Blocker™ BLOTTO (nonfat dry milk), Blocker Casein, Blocker BSA, SEA BLOCK (fish serum), SuperBlock® Blocking Buffer and StartingBlock™ Blocking Buffer. Visit our web site to obtain more information about these products.

B. Secondary Antibody Dilutions and Signal Duration

Using the appropriate dilution of HRP-conjugated secondary antibody is critical to achieving a blot with good signal-to-noise ratio and signal duration. The SuperSignal West Pico Substrate reaction results in an intense signal, and probing with secondary antibody that is too concentrated (not sufficiently dilute) will result in an overly intense signal that is quickly exhausted. Generally, experiments with SuperSignal West Pico Substrate will require much less secondary antibody than ECL Substrate. Pierce researchers usually use a 1/100,000 dilution of Pierce secondary antibodies (e.g., Product No. 31430 or 31460) when performing standard Western blot experiments.

C. Other SuperSignal West Substrates

SuperSignal West Pico Substrate is only one of several chemiluminescent substrates for HRP in the Thermo Scientific Pierce® Protein Research product line. For even greater sensitivity and 24-hour signal duration, use SuperSignal West Dura Substrate (Product No. 34075, 34076). For maximum sensitivity (low-femtogram range), use SuperSignal West Femto Substrate (Product No. 34095, 34096). Choose Pierce ECL Substrate (Product No. 32106, 32019, 32209) to obtain similar performance with lower cost using the same procedure and conditions as GE Healthcare ECL Substrate.

Related Products

34090	CL-XPosure™ Film, 5" × 7" sheets, 100 sheets/pkg
34089	CL-XPosure Film, 18 × 24 cm sheets, 100 sheets/pkg
21059	Restore™ Western Blot Stripping Buffer, 500 ml
24580	MemCode™ Reversible Protein Stain Kit for Nitrocellulose Membranes
24585	MemCode Reversible Protein Stain Kit for PVDF
21065	Pierce Background Eliminator Kit, for removing background from overexposed X-ray film
37535	SuperBlock (TBS) Blocking Buffer, 1 L
37542	StartingBlock (TBS) Blocking Buffer, 1 L
28320	Surfact-Amps® 20, 10% solution of ultrapure Tween-20 detergent, 6 × 10 ml
32110	Antibody Extender Solution NC, 500 ml, for using three times less primary antibody while maintaining signal intensity on nitrocellulose membrane
88018	Nitrocellulose Membrane, 0.45 µm, 33 cm × 3 m, 1 roll
77010	Nitrocellulose Membrane, 0.45 µm, 8 × 12 cm, 25/pkg.
88025	Nitrocellulose Membrane, 0.45 µm, 8 × 8 cm, 15/pkg.
88600	Western Blotting Filter Paper, 8 cm x 10.5 cm, 100 sheets

Tween is a registered trademark of ICI.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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