

# Pierce<sup>®</sup> Western Blot Signal Enhancer

21050

1456.3

Number	Description
21050	<b>Pierce Western Blot Signal Enhancer Kit</b> , sufficient material for 10 mini blots or 800cm <sup>2</sup> membrane <b>Kit Contents:</b> <b>Enhancer Reagent 1</b> , 250mL <b>Enhancer Reagent 2</b> , 250mL

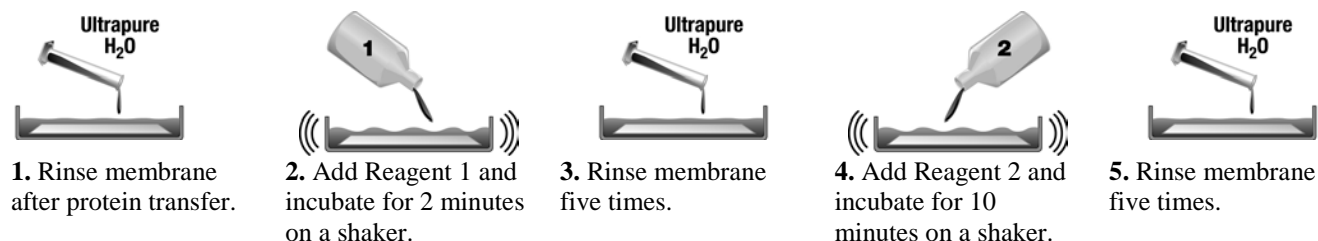
**Storage:** Upon receipt store kit at room temperature.

## Introduction

Western blotting is widely used to detect and compare proteins from a complex mixture using antibody detection on a membrane. The Thermo Scientific Pierce Western Blot Signal Enhancer Kit can significantly enhance detection of low abundance and weak immunoreactive proteins for both chromogenic and chemiluminescent signals. After proteins are transferred to either a nitrocellulose or PVDF membrane, the enhancer process requires only a few minutes to complete and the Western blot is then processed as usual.

**Note:** Signal enhancement is protein dependent and could vary from 3- to 12-fold. Treatment of proteins on PVDF membrane may not always result in signal enhancement.

## Procedure Summary



**Important:** Use Pierce Western Blot Signal Enhancer after electrotransfer of the proteins and **BEFORE** blocking nonspecific binding sites on the membrane.

## Additional Materials Required

- Ultrapure Water
- Nitrocellulose or PVDF membrane containing transferred proteins
- Rotary platform shaker for membrane agitation during incubations

## Procedure for Western Blot Enhancer Treatment

**Important Note:** For all steps, use sufficient volumes to completely immerse the blot. Enhancer treatment must be completed before blocking nonspecific sites on the membrane.

1. Use any suitable protocol for electrophoresis and protein transfer onto a nitrocellulose or PVDF membrane.
2. Rinse the membrane containing the transferred proteins by adding ultrapure water to the membrane, shaking twice and quickly decanting.
3. Add ~25mL of Enhancer Reagent 1 to the membrane. Agitate at room temperature for 2 minutes on a rotary platform shaker.
4. Rinse the membrane five times by adding ultrapure water to the membrane, shaking twice and quickly decanting.
5. Add ~25mL of Enhancer Reagent 2 to the membrane. Agitate at room temperature for 10 minutes on a rotary platform shaker.
6. Rinse the membrane five times by adding ultrapure water to the membrane, shaking twice and quickly decanting.
7. Block nonspecific sites on the membrane with a suitable blocker and continue with immunoblotting procedure to detect the protein of interest.

## Troubleshooting

Problem	Possible Cause	Solution
Weak or no signal	Low amounts or no protein present in the sample	Determine protein concentration in the original sample
	Secondary antibody concentration is too high or too low	Consult instructions for the detection system being used
Western blot resulted in high background	Inadequate blocking and/or washing	Optimize blocking buffer and washing steps
	Secondary antibody concentration is too high	Consult instructions for the detection system being used

## Additional Information

Please visit the web site for additional information including the following items:

- How to request your free copy of the 24-page Western Blotting Handbook, which contains a 10-page section on troubleshooting, detailed Western Blotting protocols and dozens of SuperSignal® Substrate references
- Tech Tip Protocol for Optimization of Antigen and Antibody Concentrations
- Tech Tip Protocol for Optimization of Blocking Buffer and Cross-Reactivity Determination
- Tech Tip Protocol for switching to SuperSignal® West Pico Substrate from ECL™ Chemiluminescent Substrate

## Related Thermo Scientific Products

26651	Chemiluminescent BlueRanger™ Molecular Weight Markers, 1 x 48 microtube plate
26681	BlueRanger™ Prestained Protein Molecular weight Marker Mix, 1 x 48 microtube plate
34080	SuperSignal® West Pico Chemiluminescent Substrate, 500mL
34076	SuperSignal® West Dura Extended Duration Substrate, 200mL
34096	SuperSignal® West Femto Maximum Sensitivity Substrate, 200mL
21059	Restore™ Western Blot Stripping Buffer, 500mL
21065	Erase-It™ Background Eliminator Kit, for eliminating background from X-ray film
34090	CL-XPosure™ Film, 5" x 7" sheets, 100 sheets/pkg

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