# do more. see more. Save more.

# Thermo Scientific Pierce ECL Western Blotting Substrate

You would never think switching ECL substrates could be so easy and save your lab so much money. Well, think again. Simply switch to Thermo Scientific<sup>™</sup> Pierce<sup>™</sup> ECL Substrate without any optimization to stretch your budget further!

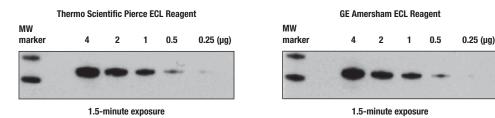


Figure 1. See any difference between these two blots? You won't find any because Thermo Scientific Pierce ECL Substrate has the same sensitivity as other entry-level ECL substrates. Dilutions of HeLa cell lysate were prepared and separated by electrophoresis. The proteins were transferred to nitrocellulose membranes (Product # 88025) and the membranes were blocked with 5% skim milk in PBS + 0.1% Tween<sup>™</sup>-20. After blocking, the membranes were incubated with Mouse Anti-Human Actin ß (US Biological, Swampscott, MA) at 1µg/mL. The membranes were washed and then incubated with 0.2µg/mL of HRP-Conjugated Goat Anti-Mouse IgG (Product # 31430) and then washed again. Working solutions of the substrates were prepared according to the manufacturers' instructions and added to the membranes for 1 minute. The membranes were removed from the substrates and placed in plastic sheet protectors. Each membrane was exposed to Thermo Scientific<sup>™</sup> CL-XPosure<sup>™</sup> Film (Product # 34090) for 90 seconds.

Figure 2. Comparison of band intensity for Thermo

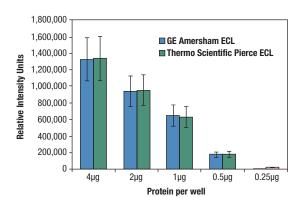
Scientific Pierce ECL Substrate vs. GE Amersham ECL

Substrate. Dilutions of HeLa cell lysate were prepared on

nitrocellulose as described in Figure 1. Error bars represent

(+/-) 20% difference in relative intensity units. Images were

scanned on a Kodak<sup>™</sup> 1D imaging station.



**Ordering Information** 

Product #	Description	Pkg. Size
32106	Pierce ECL Western Blotting Substrate Sufficient reagent for 4,000cm <sup>2</sup> of membrane	500mL kit
32209	Pierce ECL Western Blotting Substrate Sufficient reagent for 2,000cm <sup>2</sup> of membrane	250mL kit
32109	Pierce ECL Western Blotting Substrate Sufficient reagent for 400cm <sup>2</sup> of membrane	50mL kit

### **Highlights:**

- Same substrate, but for much less – about half the cost of other ECL substrates
- No optimization required truly a plug-and-play reagent – our ECL formulation is the same so there should be no need for optimization or protocol changes

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The Western Blotting Handbook and Troubleshooting Guide This guide details each step of the Western blotting process with technical information

and products. Download a PDF or request a hard copy at **thermoscientific.com/pierce** 



Western Blotting Steps and Troubleshooting Poster

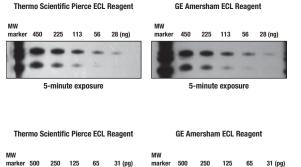
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## Thermo Scientific Pierce ECL Western Blotting Substrate



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1-minute exposure

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Figure 3. Same formulation and sensitivity between Thermo Scientific Pierce ECL Substrate and other ECL substrates allows for easy switching without any optimization! Dilutions of *E. coli* cell lysate were prepared and separated by electrophoresis. The proteins were transferred to PVDF membranes (Product # 88585) and the membranes were blocked with 5% skim milk in PBS + 0.1% Tween-20. After blocking, the membranes were incubated with Mouse Anti-ß galactosidase AB-1 (Lab Vision) at 1 $\mu$ g/mL. The membranes were washed and then incubated with 0.2 $\mu$ g/mL of HRP-Conjugated Goat Anti-Mouse IgG (Product # 31430) and then washed again. Working solutions of the substrates were prepared according to the manufacturers' instructions and added to the membrane for 1 minute. The membranes were removed from the substrates and placed in plastic sheet protectors. Each membrane was exposed to CL-XPosure Film (Product # 34090).

Figure 4. Switch to a more economical ECL substrate without wasting time on optimization! Dilutions of Recombinant Bovine TNF- $\alpha$  (Product # RBOTNFAI) were prepared and electrophoresed. The proteins were transferred to nitrocellulose membranes (Product # 88025) and the membranes were blocked with 5% skim milk in PBS + 0.1% Tween-20. After blocking, the membranes were incubated with Rabbit Anti-Bovine TNF- $\alpha$  at 4µg/mL. The membranes were washed and then incubated with 0.4µg/mL of HRP-Conjugated Goat Anti-Rabbit IgG (Product # 31460) and then washed again. Working solutions of the substrates were prepared according to the manufacturers' instructions and added to the membranes for 1 minute. The membranes were removed from the substrates and placed in plastic sheet protectors. Each membrane was exposed to Hyperfilm<sup>™</sup> Film.

#### **Complementary Products Ordering Information**

Product #	Description	Pkg. Size
24580	Pierce Reversible Protein Stain Kit for Nitrocellulose Membranes* Sufficient material to stain protein and reverse the stain from 10 (8cm x 8cm) nitrocellulose membranes.	Kit
24585	Pierce Reversible Protein Stain Kit for Polyvinylidene Difluoride Membrane* Sufficient material to stain protein and reverse the stain from 10 (8cm x 8cm) PVDF membranes.	Kit
46640	SuperSignal Western Blot Enhancer Sufficient for 25 mini blots or 2000cm <sup>2</sup> membrane.	Kit
46641	SuperSignal Western Blot Enhancer Sufficient for 2 mini blots or 160cm <sup>2</sup> membrane.	Kit
37587	Clear Milk Blocking Buffer (10X) Sufficient for 30 mini blots at 30mL each of 1X buffer.	100mL
37570	Protein-Free (TBS) Blocking Buffer Proprietary formulation in Tris-buffered saline at pH 7.4 with Kathon <sup>™</sup> Antimicrobial Agent.	1L
37571	Protein-Free T20 (TBS) Blocking Buffer Proprietary formulation in Tris-buffered saline at pH 7.4 with 0.05% Tween-20 and Kathon Antimicrobial Agent.	1L
37572	Protein-Free (PBS) Blocking Buffer Proprietary formulation in phosphate-buffered saline at pH 7.4 with Kathon Antimicrobial Agent.	1L
37573	Protein-Free T20 (PBS) Blocking Buffer Proprietary formulation in phosphate-buffered saline at pH 7.4 with 0.05% Tween-20 and Kathon Antimicrobial Agent.	1L
37538	StartingBlock <sup>™</sup> (PBS) Blocking Buffer A protein-based blocker formulation in phosphate-buffered saline (pH 7.5) for use in Western blotting and ELISA applications.	1L
37542	StartingBlock (TBS) Blocking Buffer A protein-based blocker formulation in Tris-buffered saline (pH 7.5) for use in Western blotting and ELISA applications.	1L

Product #	Description	Pkg. Size
37539	StartingBlock T20 (PBS) Blocking Buffer A protein-based blocker formulation in phosphate-buffered saline at pH 7.5 with 0.05% Tween-20 and Kathon Antimicrobial Agent.	1L
37543	<b>StartingBlock T20 (TBS) Blocking Buffer</b> A protein-based blocker formulation in Tris-buffered saline at pH 7.5 with 0.05% Tween-20 and Kathon Antimicrobial Agent.	1L
84785	<b>SuperSignal Molecular Weight Protein Ladder</b> <i>Sufficient for 50 gel lanes at 5μL per lane.</i>	250µL
34089	CL-XPosure Film (18cm x 24cm sheets)	100/pkg
34090	CL-XPosure Film (5" x 7" sheets)	100/pkg
34091	CL-XPosure Film (8" x 10" sheets)	100/pkg
34093	CL-XPosure Film (8" x 10" sheets)	50/pkg
88600	Western Blotting Filter Paper (8cm x 10.5cm sheets)	100/pkg
46428	Restore <sup>™</sup> PLUS Western Blot Stripping Buffer Sufficient reagent to strip one to two (8cm x 10cm) blots.	30mL
46430	Restore PLUS Western Blot Stripping Buffer Sufficient reagent to strip 25 (8cm x 10cm) blots.	500mL
21062	Restore Western Blot Stripping Buffer Sufficient for stripping one (8cm x 10cm) blot.	30mL
21059	Restore Western Blot Stripping Buffer Sufficient for stripping 25 (8cm x 10cm) blots.	500mL
37518	SuperBlock Blocking Buffer in PBS Sufficient reagent to block 500 membranes. This product contains Kathon CG/ICP at 600 ppm.	5L

\*\* Signal enhancement of proteins on PVDF membrane has been shown to be variable from no significant enhancement for some proteins, to several-fold enhancement for others.

To view a complete list of kit components or to view our complete line of buffers, primary and secondary detection reagents, etc. visit thermoscientific.com/western.



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