

User Guide

Immobilon® Signal Enhancer

WBSH0500

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Introduction

Immobilon® Signal Enhancer is designed for use in Western blotting applications when low signal intensity is observed. It combines signal amplification and blocking in one ready-to-use reagent. Enhancement of the antibody-antigen reaction can produce a 2 to 10-fold increase in signal intensity over traditionally used reagents. The reagent is formulated for use in both membrane blocking and antibody dilution steps and can be used for traditional immunodetection or with the SNAP i.d.® Protein Detection System. Immobilon® Signal Enhancer is compatible with PVDF as well as nitrocellulose blotting membranes and can be used with chromogenic, chemiluminescent, and fluorescent detection methods.

In applications where sufficient signal is observed, but the primary antibody availability is limited, Immobilon® Signal Enhancer can be used to reduce the amount of valuable primary antibody required.

Package Contents

Immobilon® Signal Enhancer, cat. no. WBSH0500, 500 mL

Storage/Shelf Life

Store at 2–8 °C. Refer to expiration date on product label.

Usage Guidelines

- Immobilon® Signal Enhancer must be used for blocking, as well as primary and secondary antibody dilution to ensure sufficient signal amplification.
- When using with the SNAP i.d.® Protein Detection System, refer to the user guide for detailed instructions on reagent volumes and protocol details. Immobilon® Signal enhancer replaces traditional blocking solution and antibody diluent.
- Immobilon®-FL membrane is recommended for fluorescent detection.
- Immobilon® Signal Enhancer may enhance the signal of some pre-stained molecular weight markers. It is recommended that the amount of pre-stained marker be reduced by 50-70% (example: dilute marker 1:10 in 1X sample loading buffer, loading 3–5 µL onto SDS-PAGE gel).
- Ensure that primary antibody is specific for the desired target.
- To avoid high background, always wear gloves when handling membrane.
- Handle the membrane with blunt tip forceps (cat. no. XX6200006P) to avoid tearing it.

Materials Required but not Provided

- Blotting membrane
- Primary antibody
- Labeled secondary antibody specific for primary antibody
- Wash buffer: Tris-buffered saline, pH 7.4, supplemented with Tween® 20 surfactant (TBST), or equivalent (e.g., 50 mM Tris HCl, 150 mM NaCl, 0.1% Tween® 20 surfactant)

Protocol

1. Block blotting membrane by adding sufficient Immobilon® Signal Enhancer to cover the membrane surface.
2. Incubate blot for desired length of time (typically one hour) with gentle agitation.
3. Dilute primary antibody in Immobilon® Signal Enhancer to desired concentration.
4. Remove blocking solution and add diluted primary antibody solution to the blot. Incubate blot with gentle agitation for required length of time (typically one hour).
5. Remove primary antibody solution.
6. Wash blot 3–4 times with TBST for a minimum of 5 minutes per wash.
7. Prepare secondary antibody dilution at desired concentration using Immobilon® Signal Enhancer.
8. After the last wash, discard buffer and add diluted secondary antibody solution.
9. Incubate blot for desired length of time (typically one hour) with gentle agitation.
10. Remove secondary antibody solution and wash blot 3–4 times with TBST for a minimum of 5 minutes per wash.
11. Proceed with preferred blot detection method.

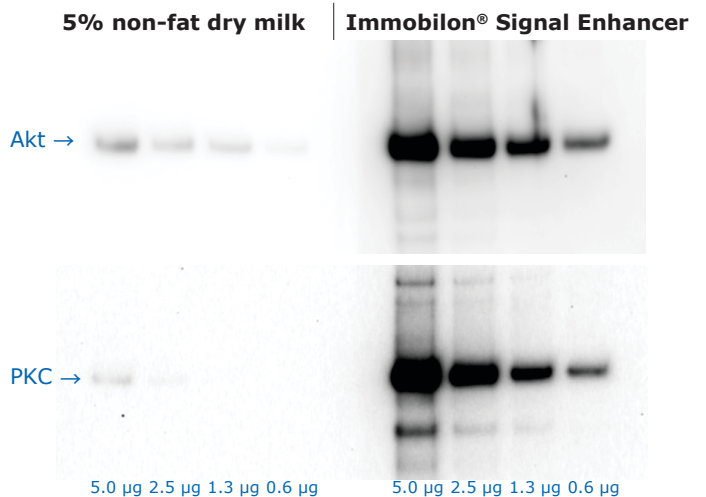
Performance

Signal amplification with Immobilon® Signal Enhancer

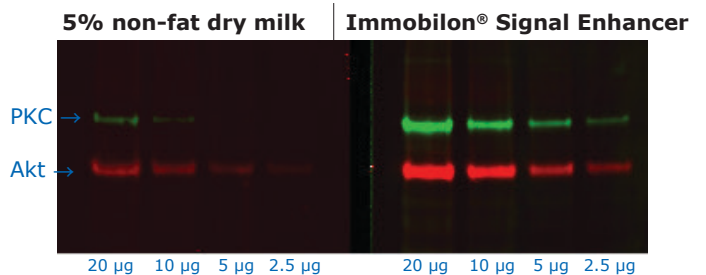
Two-fold dilution series of EGF-stimulated A431 cell lysate were resolved by SDS-PAGE and transferred onto Immobilon®-P or Immobilon®-FL membrane. Blots were blocked with either 5% non-fat dry milk or Immobilon® Signal Enhancer.

Primary antibodies (rabbit anti-Akt, cat. no. 05-796 and mouse anti-PKC antibody, cat. no. 05-983) and the labeled secondary antibodies were diluted in the respective reagents at identical dilution ratios. All blots were compared under the same conditions.

Chemiluminescent signal amplification



Fluorescent signal amplification



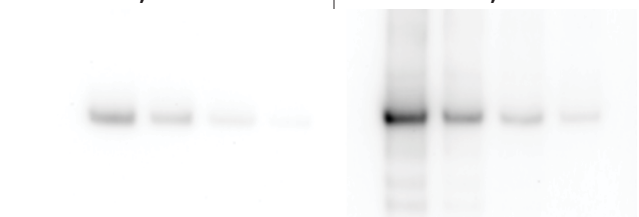
Reduction of antibody consumption

Blot A: 5% non-fat dry milk in TBST was used for blocking and antibody dilutions. Anti-Akt antibody was diluted 1:5,000.

Blot B: Immobilon® Signal Enhancer was used for blocking and antibody dilutions. The amount of primary antibody was reduced ten-fold, allowing it to be used at a dilution of 1:50,000.

Blot A: 5% non-fat dry milk, anti-Akt antibody diluted 1:5,000

Blot B: Immobilon® Signal Enhancer, anti-Akt antibody diluted 1:50,000



Product Ordering Information

Description	Qty/Pk	Cat. No.
Reagents		
Immobilon® Signal Enhancer	500 mL	WBSH0500
Blotting Membranes		
Immobilon®-E PVDF, 0.45 µm, 8.5 cm x 10 m roll	1	IEVH85R
Immobilon®-E PVDF, 0.45 µm, 26.5 cm x 1.875 m roll	1	IEVH00005
Immobilon®-E PVDF, 0.45 µm, 7 cm x 8.4 cm sheet	50	IEVH07850
Immobilon®-E PVDF, 0.45 µm, 8 cm x 10 cm sheet	10	IEVH08100
Immobilon®-P PVDF, 0.45 µm, 8.5 cm x 10 m roll	1	IPVH85R
Immobilon®-P PVDF, 0.45 µm, 26.5 cm x 3.75 m roll	1	IPVH00010
Immobilon®-P PVDF, 0.45 µm, 26.5 cm x 1.875 m roll	1	IPVH00005
Immobilon®-P PVDF, 0.45 µm, 7 cm x 8.4 cm sheet	50	IPVH07850
Immobilon®-P PVDF, 0.45 µm, 8.5 cm x 13.5 cm sheet	10	IPVH08130
Immobilon®-FL PVDF, 0.45 µm, 8.5 cm x 10 m roll	1	IPFL85R
Immobilon®-FL PVDF, 0.45 µm, 26.5 cm x 3.75 m roll	1	IPFL00010
Immobilon®-FL PVDF, 0.45 µm, 26.5 cm x 1.875 m roll	1	IPFL00005
Immobilon®-FL PVDF, 0.45 µm, 7 cm x 8.4 cm sheet	10	IPFL07810
Immobilon®-P ^{SQ} PVDF, 0.2 µm, 8.5 cm x 10 m roll	10	ISEQ85R
Immobilon®-P ^{SQ} PVDF, 0.2 µm, 26.5 cm x 3.75 m roll	1	ISEQ00010
Immobilon®-P ^{SQ} PVDF, 0.2 µm, 26.5 cm x 1.875 m roll	1	ISEQ00005
Immobilon®-P ^{SQ} PVDF, 0.2 µm, 7 cm x 8.4 cm sheet	50	ISEQ07850
Immobilon®-P ^{SQ} PVDF, 0.2 µm, 8.5 cm x 13.5 cm sheet	10	ISEQ08130
Immobilon®-NC, 0.45 µm, 8.5 cm x 10 m roll	1	HATF85R
Immobilon®-NC, 0.45 µm, 33 cm x 3 m roll	1	HATF00010
Immobilon®-NC, 0.45 µm, 7 cm x 8.4 cm sheet	50	HATF07850
Immobilon®-NC, 0.45 µm, 8.5 cm x 13.5 cm sheet	20	HATF08130
Accessories		
Filter forceps, blunt end	3	XX6200006P
Antibodies		
Primary and secondary antibodies	Go to SigmaAldrich.com	

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Disposal

Collect and dispose of used material according to all applicable international, federal, state, and local regulations.

Safety Data Sheet

Safety Data Sheets (SDS) are available at [SigmaAldrich.com](https://www.sigmaaldrich.com).

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