User Guide

Immobilon® ECL Ultra Western HRP Substrate

Cat. Nos. WBULS0100 and WBULS0500

Introduction

Immobilon® ECL Ultra Western HRP Substrate is an ultra-sensitive reagent with long signal duration for the detection of horseradish peroxidase (HRP)-labeled antibodies on western blots. The intense signal created permits sensitive detection at the mid-to-low femtogram range using x-ray film or digital imaging systems. Immobilon® ECL Ultra substrate is compatible with both PVDF and nitrocellulose blotting membranes, as well as commonly used buffers and blocking reagents.

Immobilon® ECL Ultra Western HRP Substrate is for research use only. It is not for use in diagnostic procedures.

Package Contents

Catalogue Number	Solution Volumes	Membrane Area
WBULS0100	50 mL Solution A 50 mL Solution B	Sufficient for 1000 cm²
WBULS0500	250 mL Solution A 250 mL Solution B	Sufficient for 5000 cm²

Storage/Shelf Life

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

Usage Guidelines

- Immobilon® ECL Ultra substrate is a two-part highly stable chemiluminescent substrate. Once mixed, the working solution is stable for up to one month when protected from light and stored at 2–8 °C.
- Immobilon® ECL Ultra substrate is extremely sensitive and the concentration of the primary and secondary antibody may need to be reduced for optimal signal-noise ratio.
- Bring Immobilon® ECL Ultra substrate to room temperature prior to use.
- Use of blocking buffer to dilute antibodies may reduce background and increase sensitivity.
- To avoid high background, always wear gloves when handling membrane.
- Handle the membrane with blunt tip forceps (cat. no. XX6200006P) to avoid tearing it.
- Do not use sodium azide in any blocking buffers or wash solutions, since it inhibits HRP activity.
- Prior to using this product, review the Safety Data Sheet (SDS), available at www.millipore.com.

Chemiluminescent Detection Protocol

Approximately 0.1 mL of Immobilon® ECL Ultra substrate is required per cm² of membrane area. The volumes of substrate required for some common membrane sizes are indicated below:

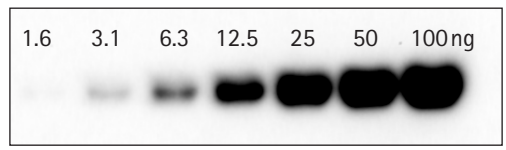
Blot Size	Immobilon® ECL Ultra Substrate Volume
7 × 8.4 cm	6 mL
10 × 10 cm	10 mL
8.5 × 13.5 cm	12 mL

1. Remove Immobilon® ECL Ultra substrate from refrigerator and allow to equilibrate at room temperature for at least 20 minutes.
2. Prepare working solution by gently mixing solutions A and B in a 1:1 ratio. Avoid vigorous agitation. Store protected from light until ready to use. Unused working solution may be stored refrigerated and protected from light for up to one month.
3. Place the blot, protein-side up, in a clean container or on a clear plastic sheet protector, and add Immobilon® ECL Ultra substrate onto the blot.
4. Incubate the blot for 2 to 5 minutes at room temperature.
5. Drain the excess substrate, transfer to a clean sheet protector, and cover the blot with plastic wrap or equivalent.
6. Visualize blots using a CCD-based imaging system or place blots into film cassette and expose to suitable x-ray film for required amount of time.

Performance

Figure 1. Detection of purified GAPDH protein

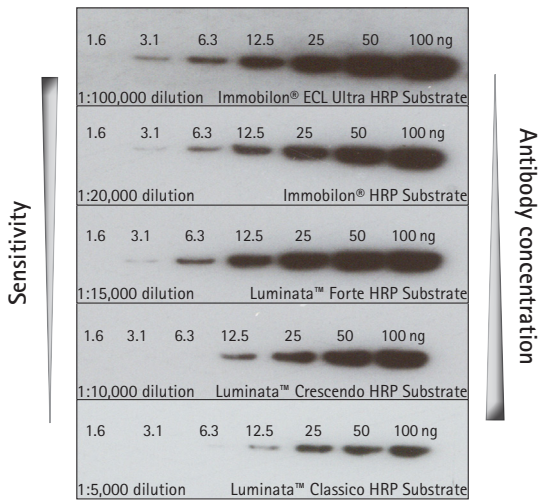
Purified GAPDH protein (Sigma® cat. no. SRE0024) was serially diluted and transferred to Immobilon®-P membrane using a fast semi-dry blotting system. Using the SNAP i.d.® 2.0 Protein Detection System, the blot was blocked with 0.5% non-fat dry milk, probed with primary antibody anti-GAPDH (cat. no. MAB374) diluted 1:100,000 and secondary antibody goat anti-mouse (cat. no. AP124P) diluted 1:10,000. The blot was then incubated with Immobilon® ECL Ultra substrate for 5 minutes and exposed to a digital imager for 30 seconds.



Performance, continued

Figure 2. Sensitivity comparison between five HRP detection reagents

Purified GAPDH protein was serially diluted (100 ng to 1.6 ng), resolved by SDS-PAGE electrophoresis, and transferred to Immobilon®-P membrane. Blots were probed with different concentrations of anti-GAPDH (cat. no. MAB374) and incubated with five different detection reagents.



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 on our web site. Go to www.millipore.com and enter your catalogue number in the search box.

Product Ordering Information

Description	Qty/Pk	Cat. No.
Immobilon® ECL Ultra Western HRP Substrate		
50 mL Solution A + 50 mL Solution B	100 mL	WBULS0100
250 mL Solution A + 250 mL Solution B	500 mL	WBULS0500
Blotting Membranes		
Immobilon®-P PVDF, 0.45 µm, 26.5 x 375 cm roll	1	IPVH00010
Immobilon®-P PVDF, 0.45 µm, 7 x 8.4 cm sheet	50	IPVH07850
Immobilon®-P PVDF, 0.45 µm, 8.5 x 13.5 cm sheet	10	IPVH08130
Immobilon®-P^{SQ} PVDF, 0.2 µm, 26.5 x 375 cm roll	1	ISEQ00010
Immobilon®-P^{SQ} PVDF, 0.2 µm, 7 x 8.4 cm sheet	50	ISEQ07850
Accessories		
Filter forceps, blunt end	3	XX6200006P
Primary and secondary antibodies	go to www.millipore.com/antibodies	

Troubleshooting

Symptom	Possible Cause	Solution
High background	Concentration of HRP-conjugated antibody too high	Increase dilution of secondary antibody.
	Inefficient blocking	Optimize blocking conditions.
	Insufficient washing	Increase wash buffer volume and /or increase number of washes.
Negative staining (white bands on black background)	Substrate depleted due to high antibody concentration	Increase antibody dilution and/or decrease antigen concentration.
Highly speckled background	High concentration of antibody and/or protein	Increase dilution of primary and secondary antibodies.
	Formation of aggregates in the HRP conjugate	Reduce protein load in the gel. Filter conjugate through 0.2 µm filter.
Nonspecific bands	High concentration of primary antibody	Increase dilution of primary antibody.
Signal disappears quickly in a blot that initially had a very high signal	High HRP-antibody concentration exhausted the substrate prematurely	Increase dilution of antibody significantly and/or decrease antigen concentration.
Weak or no signal	Antibody concentration too low	Increase exposure time. Decrease antibody dilution and/or increase antigen concentration.
	Protein did not transfer to membrane	Verify protein transfer.
	Antibody did not bind to target	Verify that antibody is specific for intended target.
	Expired substrate	Replace substrate with new lot.

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Technical Assistance

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Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms ("Conditions of Sale").