User Guide

Immobilon® UltraPlus

Western HRP Substrate

WBULP-10ML, WBULP-20ML, WBULP-100ML

Introduction

Immobilon® UltraPlus detection reagent is a non-isotopic, luminol-based chemiluminescence substrate, designed for the chemiluminescent detection of immobilized proteins conjugated with horseradish peroxidase (HRP). It is compatible with both PVDF and nitrocellulose blotting membranes, as well as commonly used buffers and blocking reagents.

Immobilon® UltraPlus substrate is for research use only. It is not for use in diagnostic procedures.

Package Contents

Solution A: Luminol/enhancer solution (amber bottle)
Solution B: Peroxide solution (white bottle)

Cat. No.	Solution Volumes	Membrane Area
WBULP-10ML	5 mL Solution A 5 mL Solution B	Sufficient for 200 cm^2
WBULP-20ML	10 mL Soltuion A 10 mL Solution B	Sufficient for 400 cm^2
WBULP-100ML	50 mL Solution A 50 mL Solution B	Sufficient for 2000 cm^2

Storage

Store at room temperature for up to one year from date of receipt.

Usage Guidelines

- Immobilon® UltraPlus substrate is a two-part highly stable chemiluminescent substrate.
- Immobilon® UltraPlus substrate is extremely sensitive and the concentration of the primary and secondary antibody may need to be reduced for optimal signal-noise ratio.
- Suggested antibody dilutions to start is 1:10,000 - 1:200,000 for primary antibody concentration and 1:300,000 - 1:1,000,000 for secondary antibody.
- 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.

Chemiluminescent Detection Protocol

Approximately 0.1 mL of Immobilon® UltraPlus substrate is required per cm^2 of membrane area. The volumes of substrate required for some common membrane sizes are indicated below:

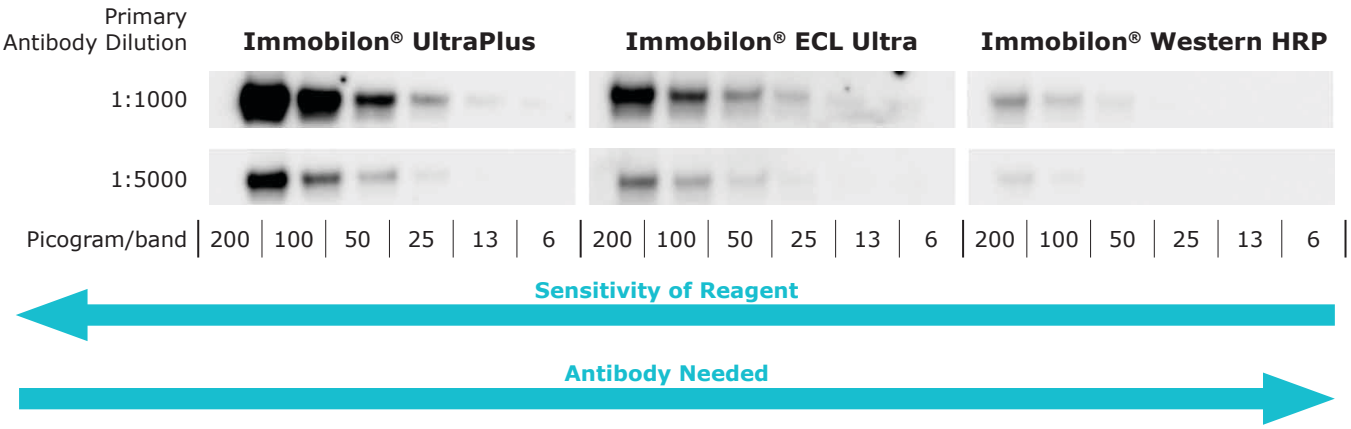
Blot Size	Immobilon® UltraPlus Substrate Volume
7 × 8.4 cm	6 mL
10 × 10 cm	10 mL
8.5 × 13.5 cm	12 mL

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.

1. Place the blot, protein-side up, in a clean container or on a clear plastic sheet protector, and add Immobilon® UltraPlus substrate onto the blot.
2. Incubate the blot for 1 to 5 minutes at room temperature.
3. Drain the excess substrate, transfer to a clean sheet protector, and cover the blot with plastic wrap or equivalent.
4. 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

Detection of Recombinant Human IKB alpha Protein (38 kDa)



Two-fold dilution series of recombinant human IKB alpha (Abcam ab113133) was resolved by SDS-PAGE and transferred to Immobilon®-P membrane using a fast semi-dry blotting system. The blots were blocked with 0.5% non-fat dry milk, probed with primary antibody rabbit anti-IKB alpha (Abcam ab32518) diluted 1:1,000 and 1:5,000 as well as secondary antibody goat anti-rabbit IgG HRP conjugated (Cat. No. AP187P) diluted 1:300,000. The blot was then incubated with Immobilon® UltraPlus substrate for 2 minutes and exposed to a digital imager for 60 seconds.

Product Ordering

Description	Membrane Area	Cat. No.
Immobilon® UltraPlus Western HRP Substrate		
5 mL Solution A 5 mL Solution B	200 cm^2	WBULP-10ML
10 mL Solution A 10 mL Solution B	400 cm^2	WBULP-20ML
50 mL Solution A 50 mL Solution B	2000 cm^2	WBULP-100ML
Blotting Membranes	Qty/Pk	
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	Qty/Pk	
Filter forceps, blunt end	3	XX6200006P

For primary and secondary antibodies go to SigmaAldrich.com/antibodies.

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 the product page at SigmaAldrich.com.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Assistance

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

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.

Notice

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