User Guide

Immobilon® Forte Western HRP Substrate

WBLUF0100, WBLUF0500

Introduction

Immobilon® Forte Western HRP Substrate is a premixed, ready-to-use reagent for chemiluminescent detection in western blotting applications that employ horseradish peroxidase (HRP)-conjugated antibodies. Immobilon® Forte Substrate is ideal for the detection of medium to low abundance proteins. It can be used with traditional immunodetection techniques or the SNAP i.d.® 2.0 Protein Detection System. Visualization of target proteins is achieved through digital imaging with a CCD camera or x-ray film.

Immobilon® Forte Package Contents

Catalog Number	Volume	Membrane Coverage
WBLUF0100	100 mL	1,000 cm^2
WBLUF0500	500 mL	5,000 cm^2

Storage/Shelf Life

Store refrigierated at 2-8 °C. Protection from light is not required. Refer to bottle label for expiration date.

Usage Guidelines

Immobilon® Forte Substrate is more sensitive than Immobilon® Classico and Immobilon® Crescendo Substrates, but very similar to the original Immobilon® Western HRP Substrate. Compared with Immobilon® Classico and Immobilon® Crescendo Substrates, Immobilon® Forte Substrate requires a lower concentration of antigen and antibodies.

See the table below for general guidelines.

Immobilon® HRP Substrate	Classico	Crescendo	Forte	Western	Ultra
Sensitivity	●	●●	●●●	●●●	●●●●●
Antibody Concentration	●●●	●●	●	●	●

Prior to using this product, review the Safety Data Sheet (SDS) to ensure awareness of associated hazards and use of appropriate controls.

Other Important Considerations

- If switching to Immobilon® Forte Substrate from a colorimetric substrate or other Immobilon® Substrate, the primary and secondary antibody concentrations may need to be decreased to achieve the optimal signal-to-noise ratio.
- Optimum exposure time to the x-ray film should be determined for each antibody system.
- Use of blocking buffer to dilute antibodies may reduce background and increase sensitivity.
- To avoid membrane tearing or high background in the blots, always wear gloves and use blunt tip forceps (XX6200006P) when handling the membrane.
- Never use objects (scissors or forceps) that show visible signs of rusting, since they may create undesirable artifacts or high background areas.
- Do not use sodium azide in any blocking buffers or wash solutions, since it inhibits HRP activity.

Chemiluminescent Detection

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

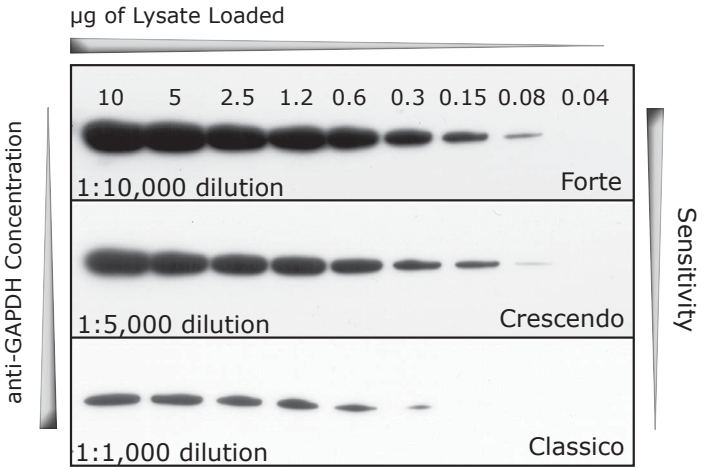
Blot Size (cm)	HRP Substrate Required
7 × 8.4	6 mL
10 × 10	10 mL
8.5 × 13.5	12 mL

1. Place the blot protein-side up in a container or clear plastic sheet protector, and add the HRP substrate onto the blot.
2. Incubate the blot for 2 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 another sheet protector.
4. Expose the blot to a suitable x-ray film for an appropriate amount of time. Typical exposure time for Immobilon® Forte Substrate is 1 to 5 minutes. The chemiluminescent signal on the blot will last for more than 1 hour. If necessary, fresh Immobilon® Forte Substrate can be added to the same blot for consecutive exposures.

Proof of Performance

Comparison of Signals from Immobilon® Forte, Crescendo, and Classico HRP Substrates

Blots of A431 cell lysates (a series of two-fold dilutions ranging from 10 µg to 0.04 µg) were prepared using Immobilon®-P membrane and probed with varying concentrations of anti-GAPDH antibody on the SNAP i.d.® 2.0 Protein Detection System. Following incubation with a goat anti-mouse HRP-conjugated secondary antibody, the blots were visualized with Immobilon® Classico, Crescendo, or Forte Substrate.



Troubleshooting

Symptom	Possible Cause	Solution
High background Negative staining (white bands on black background) Nonspecific bands	Ab concentration is too high.	Reduce exposure time. Decrease Ab concentration. Use a lower sensitivity HRP detection substrate. Decrease antigen concentration.
Signal disappears quickly in a blot that initially had a very high signal	High HRP-Ab concentration has exhausted the substrate prematurely.	Decrease Ab concentration significantly. Use a lower sensitivity HRP detection substrate. Decrease antigen concentration.
Weak or no signal	Ab concentration is too low.	Increase exposure time. Increase Ab concentration. Increase antigen concentration. Change the blocking solution.

Ordering Information

Products can be ordered from SigmaAldrich.com.

Immobilon® Western Chemiluminescent Substrates for Western Blotting Applications

Description	Qty	Catalog No.
Immobilon® Classico Western HRP Substrate	100 mL	WBLUC0100
	500 mL	WBLUC0500
Immobilon® Crescendo Western HRP Substrate	100 mL	WBLUR0100
	500 mL	WBLUR0500
Immobilon® Forte Western HRP Substrate	100 mL	WBLUF0100
	500 mL	WBLUF0500
Immobilon® Western HRP Substrate	100 mL	WBKLS0100
	500 mL	WBKLS0500
Immobilon® ECL Ultra Western HRP Substrate	100 mL	WBULS0100
	500 mL	WBULS0500

Other Western Blotting Related Products

Description	Qty	Catalog No.
Immobilon® Block-CH Chemiluminescent Blocker	500 mL	WBAVDCH01
Immobilon® Block-FL Fluorescent Blocker	500 mL	WBAVDFL01
Immobilon® Block-PO Phosphoprotein Blocker	500 mL	WBAVDP001
ReBlot™ Western Blot Recycling Kit	1	2060-M
ReBlot™ Plus Kit	1	2500

Immobilon®-E PVDF Membrane (0.45 µm pore size) for General Western Blotting Applications

Description	Qty/Pk	Catalog No.
26.5 × 187.5 cm roll	1	IEVH00005
7 × 8.4 cm sheet	50	IEVH07850
8 × 10 cm sheet	10	IEVH08100
9 × 12 cm sheet	10	IEVH09120
10 × 10 cm sheet	10	IEVH10100
Blotting Sandwich 7 × 8.4 cm	20	IESN07852
Blotting Sandwich 8.5 × 13.5 cm	20	IESN08132

Related Products for General Western Blotting Applications

Description	Qty/Pk	Catalog No.
Immobilon® blotting filter paper, 7 × 8.4 cm sheet	100	IBFP0785C
Immobilon® blotting filter paper, 8.5 × 13.5 cm sheet	100	IBFP0813C
Ponceau S solution, 0.1% (w/v) in 5% acetic acid	1 L	P7170

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