#### **User Guide**

# Immobilon®-NC Transfer Membrane

For Standard Immunodetection

HATF85R, HATF07850, HATF08130

#### Introduction

Immobilon®-NC transfer membrane is a mixed cellulose ester (mixtures of cellulose acetate and cellulose nitrate) microporous membrane used for protein blotting applications. The Immobilon®-NC membrane has a nominal pore size of 0.45 micron ( $\mu$ m) and is optimal for blotting proteins with molecular weights greater than 20 kilodaltons (kDa), Immobilon®-PSQ membrane is optimal for proteins less than 20 kDa. It is an ideal substrate for immunodetection, since it is compatible with standard blocking agents and detection protocols, including chromogenic, chemiluminescence and fluorescence. This user guide provides basic protocols for electroblotting and immunodetection using Immobilon®-NC transfer membrane.

#### Immobilon®-NC Membrane Properties and Applications

Composition Mixed cellulose esters (MCE) membrane (surfactant-free)

Pore size 0.45 µm

Applications Western Immunoblot assays

Detection methods Chemiluminescent

Chromogenic Radioactive Fluorescent\*

#### Protein visualization methods

Reversible Stains Ponceau-S, Sypro® Ruby Protein Blot Stain

Irreversible Stains Coomassie brilliant blue dye, Amido black,

India black, Colloidal gold

# **Storage Conditions and Shelf-Life**

#### Recommended for Immobilon-NC Narrow Roll (HATF85R)

Shelf life 18 months stored in a cool, dry, ventilated location

away from direct sunlight.

Storage humidity 30–70% Storage temperature 10-25 °C

#### Recommended for Immobilon-NC Midi and Mini Cut Sheets (HATF07850, HATF08130)

Nitrocellulose cut sheets are considered minimal degradation risk and therefore not considered to warrant a recommended shelf-life.

Store nitrocellulose cut sheets below 25 °C, at a relative humidity between 30–70%.



<sup>\*</sup> For fluorescence detection methods, low-autofluorescent Immobilon®-FL membrane is recommended.

### **Dispenser Box Setup**

- 1. Remove the protective bag from the membrane roll.
- 2. Lay the box so the top flap lays flat when open.
- 3. Unspool a small amount of the membrane. This is the membrane "leading edge".
- 4. Place the membrane roll in the box, being careful to orient the roll as shown.
- 5. Close the box side flaps and use the box tuck tab to secure the roll in the box.
- 6. Pull the membrane leading edge to the midi or mini blot markings on the top flap.
- 7. Cut the membrane where CUT HERE is marked on the top flap. Do not cut the top flap.
- 8. Close the top flap to store.



The Immobilon® NOW Dispenser facilitates rapid, precise measurement and cutting of transfer membranes to standard mini and midi sizes. The Immobilon® NOW Transfer Membrane Dispenser quick start guide is available online at <a href="SigmaAldrich.com">SigmaAldrich.com</a>.

Included in the narrow roll box you will find a membrane ID card along with a hazard sticker. Insert the membrane ID card into the pouch on the inside of the dispenser top. Ensure the hazard sticker is displayed in a visible location and the dispenser is stored appropriately. See SDS for details.

# Guidelines for Working with Immobilon®-NC Membrane

- Avoid fingerprints by always wearing gloves when handling the membrane
- Use blunt forceps to prevent membrane damage
- Keep the patapar (blue paper) with the membrane during cutting or handling, but discard when wetting the membrane
- Handle membrane with care to avoid scratches on the membrane surface
- Do not fold the membrane
- Immobilon®-NC membrane should be wetted with Milli-Q® water or deionized (DI) water, then equilibrated in transfer buffer before use
- After protein transfer, wash the blot with DI water to eliminate gel residues
- Blots can be used immediately or air dried and stored at 4 °C for later use

# Materials Recommended for Western Blotting

- Immobilon®-NC membrane cut to the dimensions of the gel
- Milli-Q® water or DI water
- · Orbital lab shaker
- Blot roller
- · Blunt tweezers
- Appropriately sized containers for blot incubation
- Transfer buffer optimized for the transfer method and gel type to be used
- Sheets of filter paper, thickness and quantity may vary depending on the transfer method
- Blocking buffer optimized for the membrane and antibodies to be used
- Wash buffer appropriate for the antibody being used: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05-0.1% Tween® 20 surfactant (PBST or TBST)
- Primary antibody (specific for the protein of interest), diluted in blocking buffer or wash buffer
- Secondary antibody (specific for the primary antibody), labeled with a detection enzyme (e.g., horseradish peroxidase (HRP) or alkaline phosphatase (AP)) or fluorophore, diluted in blocking or wash buffer
- Chemiluminescent or chromogenic detection reagent

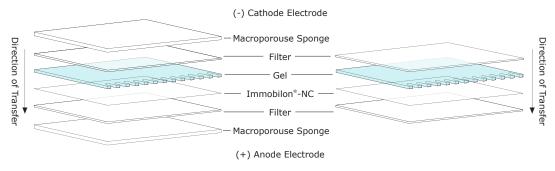
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#### **Protein Transfer Methods**

Proteins can be transferred to Immobilon®-NC membrane by two common electro-transfer methods: Wet transfer, also known as tank transfer, and semi-dry transfer.

#### **Wet Transfer Stack Assembly**

#### **Semi-dry Transfer Stack Assembly**



#### **Wet Transfer**

During wet transfer, the gel and the blotting membrane are completely submerged in the transfer buffer. Most wet transfer systems use a blot module that employs a cassette to clamp the layers of the transfer stack tightly together to ensure efficient transfer (see illustration above).

#### **Semi-Dry Transfer**

This method differs from wet transfer by using transfer buffer soaked filter papers as the ion reservoir, significantly reducing the amount of transfer buffer required. The horizontal plate electrodes significantly simplify the transfer stack set-up. Due to the short distance between the electrodes, the transfer is typically more rapid than wet transfer.

#### **Dot Blotting**

A third method of protein transfer is dot blotting, where the protein sample is directly spotted onto the blotting membrane, either manually or by using vacuum.

# **Membrane Wetting**

- 1. Add DI water to an appropriately sized container that fits the membrane to be used. Wet the membrane by carefully floating the membrane on the water for 15-30 seconds.
  - **NOTE:** Do not immerse the membrane in water or poke with a sharp object. Any dry spots (air-lock) on the membrane will inhibit the transfer. If dry spots are observed, a new membrane should be used. Wet the membrane according to the protocol. Unused membrane can be air dried overnight and stored for later use.
- 2. Equilibrate the membrane in transfer buffer for a minimum of 5 minutes.
  - **CAUTION:** Once the membrane has been wetted, do not allow it to dry out. It can be kept in buffer until protein transfer. Immobilon®-NC membrane is not compatible with alcohol (methanol, ethanol or isopropyl alcohol) concentrations above 25%.

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#### Western Blotting with Immobilon®-NC Membrane

- 1. Resolve the protein mixture on a 1D or 2D polyacrylamide gel.
- 2. Immerse the gel in the transfer buffer and allow it to equilibrate for 10–15 minutes. For best results it is recommended that the transfer buffer is prepared fresh.

#### **Wet Transfer Method**

- 1. Wet two macroporous sponges and two pieces of filter paper in transfer buffer for each gel to be transferred.
- 2. Place wet transfer module in the tank of the transfer system.
- 3. Assemble transfer stacks according to system instructions and place into blot module. Depending on the wet transfer system, an ice pack may be used for cooling of the transfer buffer. Alternatively, cold buffer can be used, or the whole process may be performed in a cold room.

**CAUTION:** To avoid air locking and ensure an even transfer, remove air bubbles by carefully rolling a clean pipette or blot roller over the surface of each layer in the stack. Do not apply excessive pressure, as this may damage the gel and membrane.

- 4. Add the required amount of transfer buffer ensuring the blotting assembly is covered with buffer.
- 5. Connect the wet transfer system to a power supply.
- 6. Follow the transfer system instructions to transfer proteins (optimization may be required).
- 7. Remove the blot from the transfer system and rinse the membrane briefly in DI water to remove gel debris. The blot may be used immediately for the immunodetection step, or it may be air dried and stored for later use.

**NOTE:** Drying the blot before immunodetection may enhance the binding of some proteins and reduce background noise.

#### **Semi-dry Transfer Method**

- 1. Wet the required filter paper in transfer buffer.
- 2. Assemble the transfer stack as instructed in the transfer system user guide.

**CAUTION:** To avoid air locking and ensure an even transfer, remove air bubbles by carefully rolling a clean pipette or blot roller over the surface of each layer in the stack. Do not apply excessive pressure, as this may damage the gel and membrane.

- 3. Connect the transfer system to a power supply
- 4. Follow the transfer system instructions to transfer proteins (optimization may be required).
- 5. Remove the blot from the transfer system and rinse the membrane briefly in DI water to remove gel debris. The blot may be used immediately for the immunodetection step, or it may be air dried and stored for later use.

**NOTE:** Drying the blot before immunodetection may enhance the binding of some proteins and reduce background noise.

#### **Protein Visualization** (Optional)

To visualize the protein transfer efficiency, Immobilon®-NC membrane may be stained with any reversible blot stain compatible with immunodetection (e.g., Ponceau-S or Sypro® Ruby Protein Blot Stain). Proteins may be analyzed by immunostaining, glycoprotein staining or mass spectrometry immediately after staining. It is important to photograph or otherwise document the Sypro® Ruby Protein Blot Stain before immunostaining, as over 90% of the stain is washed off the blot during the blocking step.

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#### Immunodetection with Immobilon®-NC Membrane

Immunodetection is an antibody-based method that allows for the detection, identification, and quantification of a protein or antigen on the blotting membrane. For best results, proper optimization of all detection parameters, such as sample amount, blocking reagent, primary and secondary antibody dilution and in the case of chemiluminescent detection, the sensitivity of the detection reagent. The typical protocol follows these six general steps:

- Carefully wet membrane with DI water. Blot should be covered with liquid during blocking, primary & secondary antibody incubation and all wash steps.
- 2. Block unoccupied membrane sites with a compatible blocking agent to prevent nonspecific binding of antibodies.
- 3. Incubate the membrane with a primary antibody that binds to the protein of interest.
- 4. Wash the membrane to remove any unbound primary antibody.
- 5. Incubate the membrane with a labeled secondary antibody which binds to the primary antibody.
- 6. Wash to remove any unbound secondary antibody.
- 7. Incubate the membrane with a chromogenic or chemiluminescent substrate that reacts with the conjugated secondary antibody to visualize protein of interest.

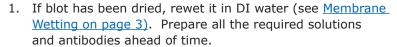
#### **Standard Immunodetection**

- 1. If blot has been dried, rewet membrane in DI water, see Membrane Wetting on page 3.
- 2. Place the blot in 0.2 mL of blocking buffer per cm<sup>2</sup> of membrane, and incubate for 1 hour with gentle agitation.
- 3. Dilute the primary antibody in 0.2 mL of wash or blocking buffer per cm<sup>2</sup> of membrane.
- 4. Remove blocking buffer.
- 5. Add diluted primary antibody solution to blot and incubate for 1 hour with gentle agitation.
- 6. Wash the blot with 0.5-1 mL of wash buffer per cm<sup>2</sup> of membrane (tris- or phosphate-buffered saline solution, supplemented with Tween® 20 surfactant (TBST or PBST)) 3–5 times for 5 minutes each wash.
- 7. Prepare enzyme or fluorescently labeled secondary antibody in 0.2 mL of wash or blocking buffer per cm² of membrane.
- 8. Add diluted secondary antibody solution to the blot and incubate for 1 hour with gentle agitation.
- 9. Wash the blot with 0.5-1 mL of wash buffer per cm<sup>2</sup> of membrane, 3-5 times for 5 minutes each wash.
- 10. Place the blot into a clean container and add 0.1 mL of the appropriate detection reagent per cm<sup>2</sup> of membrane (chemiluminescent (Alkaline phosphatase (AP) or horseradish peroxidase (HRP), or chromogenic). For fluorescent detection, skip to step 12.
- 11. Incubate 1–5 minutes, according to the detection reagent manufacturer's instructions.
- 12. Detection
  - For HRP or AP chemiluminescent reagents, expose blot to x-ray film or acquire the image using a digital imaging system
  - For chromogenic detection, add the reagent and wait until signal appears
  - For fluorescent detection, image the blot with appropriate scanning conditions (excitation, emission, resolution and intensity) according to fluorescent dye conjugated to the secondary antibody)

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#### SNAP i.d.® 2.0 Immunodetection Using Vacuum Filtration

The SNAP i.d.® 2.0 system uses vacuum to drive blocking reagents, primary and secondary antibody solution, and wash buffers directly through the blotting membrane. This unique vacuum-driven system significantly reduces the length of time required for immunodetection. What previously took 4 to 24 hours with traditional Western blotting methods now takes only 30 minutes with no loss of signal intensity or reduction in blot quality.



**NOTE:** Antibodies should be 3 to 5 times more concentrated than in standard immunodetection, but in volumes of 2.5–10 mL, depending on the blot size/blot holder.

- 2. Wet the SNAP i.d.® blot holder and assemble the blot with the protein side down.
- 3. Using the blot roller, remove all air bubbles and excess water, and insert the blot holder inside the SNAP i.d.® frame.
- 4. Block by adding 15–30 mL of blocking solution and immediately turn the vacuum on.
- 5. When all blocking solution has been removed, turn the vacuum off.
- 6. Depending on the blot holder size used, add 2.5–10 mL of diluted primary antibody and incubate for 10 minutes.
- 7. Turn vacuum on to flush the antibody, then with the vacuum still on, wash 4 times with 15–30 mL of wash buffer.
- 8. Turn vacuum off, add 2.5–10 mL (depending on the blot holder size used) of diluted secondary antibody, and incubate for 10 minutes.
- 9. Turn vacuum on to flush the antibody, then wash 4 times with 15–30 mL of wash buffer.
- 10. Turn the vacuum off.
- 11. Remove the blot from the blot holder and continue with the detection method of choice (chemiluminescence or chromogenic).



#### **Time Comparison**

Standard immunodetection takes at least 4 hours where the SNAP i.d.® 2.0 Protein Detection System can perform the same process with significant time savings.

	Standard	SNAP i.d.® 2.0
Block membrane	1 hour	10 seconds
Incubate with primary antibody	1 hour	10 minutes
Wash membrane	3 times, 5 minutes each	4 times, ~10 seconds each
Incubate with secondary antibody	1 hour	10 minutes
Wash membrane	3 times, 5 minutes each	4 times, ~10 seconds each
Total time	4 hours	22 minutes

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# **Troubleshooting**

# **General Western Blotting**

Problem	Cause	Solution
Immobilon®- NC	Air locking has occurred	Never pour water on top of or immerse the membrane in DI water; always add DI water to a dish first, than float membrane on surface
membrane has dry spots	Air bubble trapped under membrane wetting	Ensure air is not trapped when adding membrane to DI water
Immobilon®-NC membrane was dissolved or shrunk	100% alcohol was used for membrane wetting	Wet membrane in DI water only; membrane is not compatible with alcohol concentrations $>\!25\%$
Poor detection by trans-illumination	Membrane not compatible with trans-illumination	Use reversible protein stain, such as Ponceau S
Uneven staining	Uneven wetting of Immobilon®-NC membrane	Follow the directions for Wet Transfer on page 3
	Immobilon®-NC membrane not equilibrated in transfer buffer	After wetting membrane with DI water, equilibrate in transfer buffer for a minimum of 5 minutes
	Membrane sandwich was incorrectly assembled	Membrane should be facing the anode, refer to <u>Protein Transfer</u> <u>Methods on page 3</u> , use prestained markers to monitor transfer
	Transfer time too short	Optimize transfer time for protein of interest
No protein detected after transfer	Electric field incorrect (too high or too low)	Optimize transfer condition for protein of interest
	Proteins blew through the membrane	Optimize transfer buffer composition (ie alcohol/ SDS concentration)
	Protein flowed in wrong direction	Ensure electrode leads are connected red to red and black to black
	Antibody concentration too low	Increase concentration of primary and secondary antibodies
	Azide added to reagents	HRP-labeled antibodies should not be used in solutions containing sodium azide
	Primary antibody was raised against native protein	Separate proteins in non-denaturing gel or use antibody raised against denatured antigen
Large splotches	Fold marks or forceps imprints on the blot	Avoid folding membrane; use only blunt end forceps
detected on blot	Fingerprints on blot	Always wear clean gloves when handling the membrane, never touch membrane with without gloves
No protein detected in same areas of the blot	Air bubble trapped between blot sandwich layers	Use a blot roller to gently remove bubbles during blot sandwich assembly
	Too many blots per container	Do not incubate and wash more than one blot per container
Uneven, splotchy	Not enough volume of solution added during incubation steps	Ensure that blot is covered with a sufficient amount of solution during all incubation steps
background	Protein side of blot is facing bottom of container	Incubate blot with the protein side facing up to ensure efficient exposure to all solutions
	Aggregates in the blocking reagent	Filter blocking reagent solution through 0.2 µm or 0.45 µm filter
Speckled background	Aggregates in HRP-conjugated secondary antibody	Filter secondary antibody solution through 0.2 µm or 0.45 µm filter
Dackground	Residue from powdered gloves transfered to blot	Only use powder free gloves

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Problem	Cause	Solution
	Membrane not uniformly wetted	Wet membrane by floating on top of DI water for 30 seconds. Refer to Membrane Wetting on page 3
	Air bubbles trapped between layers of blot sandwich stack	Use a blot roller to gently remove bubbles during blot sandwich assembly
	Uneven contact between gel and membrane	Use blot roller to ensure gel and membrane surfaces have uninterrupted contact
	Too much heat generated during the wet transfer	Pre-chill the buffer, add an ice pack to the transfer tank, or perform the transfer in a cold room
Band smeared/	Too much heat generated during semi dry transfer	Shorten the run time, increase the number of filter papers, or reduce the voltage/current
distorted	Filter paper dried out during	Make sure filter paper is thoroughly saturated with transfer buffer prior to transfer or use additional sheets
	semi-dry transfer	Use additional filter paper sheets
		Transfer sandwich can not be stored and must be assembled immediately before starting the transfer
	Proteins transferred too rapidly Protein buildup on the membrane surface	Reduce the strength of the electrical field
	Electric field too high	Refer to equipment or gel manufacture's recommendations, optimize voltage/amperage for protein of interest
	Poor reagent quality or reagent contamination	All of the buffers and reagents should be prepared fresh
	Nonspecific protein binding to the membrane	Ensure the use of clean electrophoresis equipment and components along with high-quality reagents
	Membrane blocking requires optimization	Verify blocking reagent is compatible with the antibody/detection system used
	Insufficient washes	Increase washing volumes and times
High background	Secondary (enzyme-conjugated) antibody concentration is too high	Increase antibody dilution
	Protein-protein interactions	Use Tween®-20 (0.1%) in the wash and antibody solutions to minimize protein-protein interactions and increase the signal to noise ratio
	Cross-reactivity between blocking reagent and antibody	Use different blocking agent or use Tween®-20 detergent in the washing buffer
	Overexposure	Shorten exposure time
	Membrane drying during incubation process	Use volumes sufficient to cover the membrane during incubation
	Poor-quality antibodies	Use high-quality affinity purified antibodies
	Detection reagent is too sensitive	Optimize detection reagent selection
	Excess detection reagents	Drain blots completely before exposure
Membrane damage	Membrane shrunken or dissolved	Ensure alcohol concentrations > 25% are not used
	Primary antibody concentration too high	Increase primary antibody dilution
Nonspecific	Secondary antibody concentration too high	Increase secondary antibody dilution
binding	Antigen concentration too high	Decrease amount of protein loaded on the gel
	Membrane was not blocked prior to addition of primary antibody	Ensure a blocking reagent compatible with the antibody/detection system used
Poor transfer of positively charged proteins	Protein net charge in the transfer buffer is positive; proteins move toward cathode	Reverse the transfer stack such that the membrane is on the cathode side of the gel
Reverse images on film (white bands on dark background)	Too much HRP-conjugated secondary antibody	Reduce concentration of secondary HRP-conjugated antibody

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High overall	High hackground fluorescence	
background	High background fluorescence from the blotting membrane	Use Immobilon®-FL PVDF blotting membrane
when using fluorescence for fluorescence detection Blocking reagents not optimized for fluorescence detection		Use Immobilon® Block-FL (Fluorescent Blocker), a noise-canceling reagent optimized for fluorescent Western blots
Multiplexing experiment did not work	Two antibodies with different fluorophores were used but host species was the same	The two antibodies must be derived from different host species; use cross-adsorbed secondary antibodies in two-color detection
		Reduce voltage by as much as 50%
	Proteins passing through the -	Equilibrate the gel in the transfer buffer for at least 15 minutes
	membrane	Increase the alcohol to $10-20\%$ , especially for smaller molecular weight proteins
		Switch to Immobilon®-PSQ transfer membrane
	_	Reduce the alcohol concentration in the transfer buffer
	_	Increase SDS concentration (0.01%–0.05%) in the transfer buffer
	Proteins retained in the gel, incomplete protein transfer	Stain gel post transfer to optimize transfer condition and transfer buffer composition
	_	Increase transfer time
		Increase electrical field
	Isoelectric point of the protein is at, or close to, the pH of the transfer buffer	To facilitate transfer, try a higher pH buffer such as 10 mM CAPS buffer at pH 11, including 10% alcohol or a lower pH buffer such as an acetic acid buffer
	Poor detection when urea is used in the gel and/or transfer buffer	Reduce the temperature by using a circulating buffer
	Poor transfer of low molecular	Switch to Immobilon®-PSQ transfer membrane
	weight proteins	Remove SDS from the transfer buffer
	Insufficient protein binding time	A lower voltage may optimize binding of small proteins to the membrane
Weak or low	Current bypasses the gel stack during semi dry transfer	Make sure the gel is not larger than the blotting paper or the membrane
signal	Improper blocking reagent	Try a different blocking agent or blocking agent concentration
	Insufficient antibody reaction time	Increase the antibody incubation time
	Antibody concentration is too low or antibody is inactive	Increase antibody concentration or prepare fresh
	Outdated detection reagents	Use fresh substrate and store properly
	Tap water inactivates chromogenic detection reagents	Use Milli-Q® water or DI water for reagent preparation
	Antibody not compatible with Western blotting	Check that antibody is suitable to be used in Western blot application
	Azide was added to reagents	Azide inhibits HRP; HRP-labeled antibodies should not be used without solutions containing sodium azide
	Fluorescent image was acquired while blot was wet	Drying the blot may enhance signal strength
	Fluorescent blot photo-bleached during incubation	Protect the membrane from light during secondary antibody incubations and washes
	Detection reagent is not sensitive enough	Optimize detection reagent selection
	Insufficient signal amplification	Antibody not suitable for Western blotting or compatible with preparation of cells/tissue
	Too few antibody binding sites	preparation or cells/ussue
	Wrong excitation wavelength	Follow dye manufacturer's instructions for blot imaging

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# **Dot and Slot (Filtration) Blotting**

Problem	Cause	Solution
		Ensure the blot sandwich transfer stack is closed properly
Slow or no	Inadequate vacuum	Ensure the vacuum source (e.g. pump) is operating properly
filtration of the sample through		Seal off any open wells with a high-quality laboratory tape
the membrane	Membrane pores clogged	Centrifuge or filter sample to remove particulates and dilute viscous samples with buffer
Little or no	Not enough protein applied to the membrane	Minimize sample dilution
protein observed on the blot  Detergents (e.g.,	Detergents (e.g., SDS) may inhibit proteins from binding to the membrane	Eliminate detergents if possible
	Air bubbles trapped in the interior of the membrane	Refer to Membrane Wetting on page 3
Stained blot is	Air bubbles in the sample	Carefully pipette samples into well to avoid the formation of air bubbles
not uniform	Not enough sample volume loaded	Sample must cover the entire exposed membrane area
	Sample leaked across the wells	Make sure the blot sandwich transfer stack is properly assembled, closed and sealed prior to filtration
Protein smeared across the top of the membrane	Membrane capacity was exceeded	Reduce the amount of protein loaded into the well

# **Safety Data Sheet**

Safety Data Sheets (SDS) are available on the product page at <u>SigmaAldrich.com</u>. Scroll down to the Documentation section.

# **Symbol Definitions**

	Flammable
NON	Non-sterile
(2)	Single Use Only
REF	Catalogue Number
LOT	Lot Number
<b></b>	Manufacturer

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# **Guidelines for Choosing an Immobilon® Blotting Membrane**

The following table provides general guidelines for choosing the appropriate membrane for a specific post-Western blot application. Due to variations in protein properties such as charge density, conformation, and hydrophobicity, not all proteins behave the same way on a given membrane surface. Experiments with a variety of Immobilon® membranes may be necessary to optimize results for your specific application.

Application after Western blotting	Membrane of choice for most proteins
General immunodetection	Immobilon®-NC Immobilon®-E Immobilon®-P
Amino acid analysis	Immobilon®-P
Immunodetection and sequencing of low molecular weight or low-abundance proteins	Immobilon®-P <sup>SQ</sup>
Fluorescence immunodetection and chemifluorescence methods	Immobilon®-FL

# **Product Ordering**

Purchase products online at SigmaAldrich.com.

Transfer Membranes and Blotting Paper Immobilon® Blotting Filter Paper sheet, 7 cm x 8 .4 cm 100 IBFP0785C  Immobilon®-E Blotting Sandwich sheet, 7 cm x 8.4 cm 20 IESN07852  Immobilon®-E PVDF Membrane roll, 26.5 cm x 1.875 m 1 IEVH00005	Description	Qty	Catalogue Number
sheet, 7 cm x 8 .4 cm 100 IBFP0785C  Immobilon®-E Blotting Sandwich sheet, 7 cm x 8 .4 cm 20 IESN07852  Immobilon®-E PVDF Membrane	<b>Transfer Membranes and</b>	Blotting	Paper
Immobilon®-E Blotting Sandwich sheet, 7 cm x 8.4 cm 20 IESN07852 Immobilon®-E PVDF Membrane	Immobilon® Blotting Filter Pap	er	
sheet, 7 cm x 8.4 cm 20 IESN07852  Immobilon®-E PVDF Membrane	sheet, 7 cm x 8 .4 cm	100	IBFP0785C
Immobilon®-E PVDF Membrane	Immobilon®-E Blotting Sandwic	h	
	sheet, 7 cm x 8.4 cm	20	IESN07852
roll, 26.5 cm x 1.875 m 1 IEVH00005	Immobilon®-E PVDF Membrane		
	roll, 26.5 cm x 1.875 m	1	IEVH00005
roll, 8.5 cm x 10 m 1 IEVH85R	roll, 8.5 cm x 10 m	1	IEVH85R
sheet, 7 cm x 8.4 cm 50 IEVH07850	sheet, 7 cm x 8.4 cm	50	IEVH07850
Immobilon®-FL PVDF Membrane	Immobilon®-FL PVDF Membrane	е	
roll, 26.5 cm x 1.875 m 1 IPFL00005	roll, 26.5 cm x 1.875 m	1	IPFL00005
roll, 26.5 cm x 3.75 m 1 IPFL00010	roll, 26.5 cm x 3.75 m	1	IPFL00010
roll, 8 .5 cm x 10 m 1 IPFL85R	roll, 8 .5 cm x 10 m	1	IPFL85R
sheet, 7 cm x 8.4 cm 10 IPFL07810	sheet, 7 cm x 8.4 cm	10	IPFL07810
Immobilon®-P Blotting Sandwich	Immobilon®-P Blotting Sandwic	:h	
sheet, 7 cm x 8.4 cm 20 IPSN07852	sheet, 7 cm x 8.4 cm	20	IPSN07852
Immobilon®-P PVDF Membrane	Immobilon®-P PVDF Membrane		
roll, 26.5 cm x 1.875 m 1 IPVH00005	roll, 26.5 cm x 1.875 m	1	IPVH00005
roll, 26.5 cm x 3.75 m 1 IPVH00010	roll, 26.5 cm x 3.75 m	1	IPVH00010
roll, 8.5 cm x 10 m 1 IPVH85R	roll, 8.5 cm x 10 m	1	IPVH85R
sheet, 7 cm x 8.4 cm 20 IPSN07852	sheet, 7 cm x 8.4 cm	20	IPSN07852
sheet, 7 cm x 8.4 cm 50 IPVH07850	sheet, 7 cm x 8.4 cm	50	IPVH07850

Description	Qty	Catalogue Number	
Immobilon®-PSQ PVDF Membra	ne		
roll, 26.5 cm x 1.875 m	1	ISEQ00005	
roll, 26.5 cm x 3.75 m	1	ISEQ00010	
roll, 8.5 cm x 10 m	1	ISEQ85R	
sheet, 7 cm x 8.4 cm	50	ISEQ07850	
Immobilon®-NC Transfer Membrane			
roll, 33 cm x 3 m	1	HATF00010	
roll, 8.5 cm x 10 m	1	HATF85R	
sheets, 7 cm x 8.4 cm	50	HATF07850	
sheets, 8.5 cm x 13.5 cm	20	HATF08130	
Immobilon® NOW Dispenser	1	IMDISP	
<b>Transfer Systems</b>			
mPAGE®	1	MWTS	
Mini Wet Transfer System			
Includes: Tank (1), Tank Lid with Electrode Cables (1),			
Mini Wet Transfer Module (1), Mini Wet Transfer Cassettes			
(2), Macroporous Sponges (5), and Freezer Packs (2)			

#### **Power Supplies**

	US Plug	MA400-US
Basic power supply for protein and DNA electrophoresis	Euro plug	MA400-EU
	UK plug	MA400-UK
	Japan plug	MA400-NI
	China plug	MA400-ZH
	US Plug	MA700-US
High capacity power supply for electrophoresis and Western blotting	Euro plug	MA700-EU
	UK plug	MA700-UK
	Japan plug	MA700-NI
	China plug	MA700-ZH

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Description	Qty	Catalogue Number
Immunodetection Device	es	
SNAP i.d.® 2.0 Systems		
Mini, 7.5 cm x 8.4 cm	2	SNAP2MINI
MultiBlot, 4.5 cm x 8.4 cm	2	SNAP2MB3
Mini, 7.5 cm x 8.4 cm and MultiBlot, 4.5 cm x 8.4 cm	1 pk	SNAP2MB1
Blocking, Enhancing and Stripping Reagents		
Immunoblot Blocking Reagent	20 G	20-200
ChemiBLOCKER™	2x 500 mL	2170
5% Alkali-soluble Casein	225 mL	70955
Tris Buffered Saline	1 L	T5912
TWEEN® 20, for molecular	50 mL	P9416
biology, viscous liquid	100 mL	P9416
Immobilon® Block-PO Reagent,	100 mL	WBAVDP001 -100ML
Phosphoprotein Detection	500 mL	WBAVDP001
Immobilon® Block-FL Reagent,	100 mL	WBAVDFL01 -100ML
Fluorescent Detection	500 mL	WBAVDFL01
Immobilon® Block-CH Reagent, Chemiluminescent Detection	100 mL	WBAVDCH01 -100ML
Chemiuminescent Detection	500 mL	WBAVDCH01
Western Blocker™ Solution	400 mL	W0138
Immobilon® Signal Enhancer	100 mL	WBSH0500 -100ML
for Immunodetection	500 mL	WBSH0500
Blot Restore Membrane Rejuvenation Kit, 10x  • Solution A, 50 mL  • Solution B, 50 mL	1	2520-M
Re-Blot™ Plus Strong Antibody Stripping solution, 10X	2x 25 mL	2504
Western-Re-Probe Reagent	100 mL	WB59

Description	Qty	Number

#### **Western Blotting Detection Reagents**

Immobilon® Ultra Plus Western HRP Substrate	20 mL	WBULP-20ML
	100 mL	WBULP-100ML
Immobilon® ECL Ultra Western HRP Substrate	20 mL	WBULS0100- 20ML
	100 mL	WBULS0100
Immobilon® Western Chemiluminescent HRP Substrate	2x 50 mL	WBKLS0100
	2x 250 mL	WBKLS0500
Immobilon® Forte Western HRP Substrate	100 mL	WBLUF0100
	500 mL	WBLUF0500
Immobilon® Crescendo Western HRP Substrate	100 mL	WBLUR0100
	500 mL	WBLUR0500
Immobilon® Classico Western HRP Substrate	100 mL	WBLUC0100
	500 mL	WBLUC0500

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