VerdiBlot Membrane – User Guide

Version 2.0

Contents:

1- Introduction

VerdiBlot is a universal Western blot membrane that ensures exceptional protein-binding capacity, superior mechanical strength, and ultra low background in chemiluminescent and fluorescent detection.

VerdiBlot was designed to merge the high sensitivity and durability of PVDF with the low background of nitrocellulose. Also, it is fully compatible with third party equipment for wet and semi-dry transfer, (more details in Section 4 of this Guide).

This Guide provides an expanded and detailed overview of the VerdiBlot workflow – from membrane activation and transfer setup to detection, re-probing, and troubleshooting. Follow the outlined steps to ensure maximum performance and reproducibility.

⚠ Always wear clean, powder-free gloves to prevent contamination and handle membranes only with forceps, preferably with blunt tip to avoid excessive marking on the membrane.

2- Properties

Table 1. VerdiBlot properties

Material	Polycaprolactone
Pore size	0.3 µm
Membrane type	Hydrophobic
Protein size	>10 kDa
Protein stain compatibility	Ponceau S Coomassie Blue Colloidal gold Others
Detection methods	Chemiluminescent Fluorescent (ultra-low background) Chromogenic
Applications	Western blot (wet and semi-dry method) Dot blotting Mass Spectrometry
Storage	Store membranes dry, in a dust-free environment, at room temperature away from direct sunlight.
Membrane activation/ wetting	The membrane can be equilibrated in transfer buffer containing at least 10% ethanol or methanol for a 3-5 minutes. In cases were the above activation is insufficient (rare, observed as uneven wetting of membrane) activate the membrane by immersing it in 100% ethanol for a few seconds, then equilibrate it in transfer buffer for few minutes.

For more complete information refer to the Technical Data Sheet found at verdiblot.com

3- Protein Transfer Setup

The following is an example of a standard western blot setup, which will be suitable for the vast majority of users. In some cases, there might be the need to optimize some parameters (buffer, running time, etc.).

Compatibility with equipment

VerdiBlot membrane is fully compatible with all wet transfer systems currently in the market. There are no special precautions when setting up your experiment

Buffer

Prepare the appropriate transfer buffer according to your system. Use the standard buffer formulation routinely employed in your laboratory, ensuring that it contains 10–20% methanol or ethanol to enhance protein binding and minimize diffusion during transfer. A commonly used buffer is:

Towbin buffer (for wet transfer): 25 mM Tris, 192 mM glycine, 20% (v/v) methanol, pH 8.3

Assembly of the Transfer Sandwich:

Before assembling the transfer sandwich, ensure that all components are **fully equilibrated in transfer buffer** to prevent drying or uneven contact. Follow the steps below and refer to Figure 1 for schematic guidance.

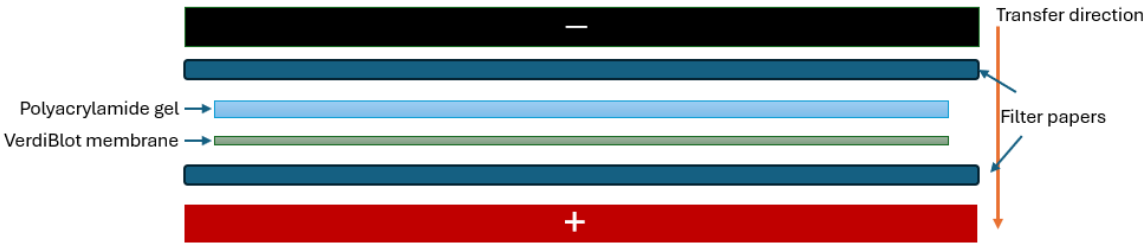

Fig. 1 In the schematic, note the order of components cathode to anode: sponge → filter paper → gel → VerdiBlot membrane → filter paper → sponge. Ensure the membrane faces the positive electrode (anode) to drive negatively charged proteins out of the gel onto the membrane.

Typical procedure for assembling transfer sandwich for wet transfer:

a) **Sponge layer**: Soak the blotting sponge thoroughly in the transfer buffer and place it at the bottom of the transfer cassette.

b) **Filter paper:** Place a sheet (thick or several thin layers) of filter paper, pre-soaked in transfer buffer, directly on top of the sponge.
c) **VerdiBlot membrane:** After equilibrating the membrane in transfer buffer containing >10% EtOH or MeOH for a 3-5 min, carefully place the membrane on top of the filter paper - avoid trapping air bubbles. In cases where the above activation is insufficient (rare) activate the membrane by immersing it in 100% ethanol for a few seconds, then equilibrate it in transfer buffer for 5–10 minutes.
d) **Gel:** Following electrophoresis, equilibrate the gel for 5–10 minutes in transfer buffer to remove excess SDS. Gently place the gel on top of the membrane, ensuring perfect alignment.
e) **Top filter paper:** Add a second pre-soaked filter paper layer above the gel. Remove air bubbles by gently rolling a clean glass tube or roller across the stack.
f) **Top sponge:** Place another transfer sponge (fully soaked in buffer) on top to complete the sandwich stack.
g) **Assembly:** Close the cassette firmly and insert it into the wet transfer chamber, ensuring correct orientation (membrane toward the anode, gel toward the cathode). Proceed with the standard wet transfer program used in your workflow.

Transfer Protocol

Once the transfer sandwich has been assembled and correctly positioned in either the wet transfer chamber or the semi-dry cassette, set the current and voltage parameters according to the table below. Always verify that the orientation of the membrane (toward the anode) and gel (toward the cathode) is correct before starting the run.

System	Current/Voltage	Transfer Time
Wet Transfer	30–90 V	60–120 min

⚠ Exceeding recommended voltage settings can cause buffer heating, membrane warping, or excessive protein diffusion. Start with recommended settings.

❄ Use pre-chilled transfer buffer and, if possible, cooling blocks or an ice pack to minimize heat generation during long transfers.

Ensure the transfer stack is fully saturated and free of air bubbles before starting the run. Even small air pockets can cause uneven protein transfer and artifacts.

For wet transfers, monitor the temperature periodically, especially for long runs. Replace warm buffer if necessary.

Blocking

Blocking is essential to prevent non-specific antibody binding on membranes.

Suitable blocking agents include:

- 5 % non-fat dry milk
- 3–5 % bovine serum albumin (BSA)
- Casein (often ~1–3 %)

Typically, the blocking solution is prepared in TBS or PBS (often with 0.05-0.2 % Tween-20) and incubate the membrane for 1 hour at room temperature, or overnight at 4 °C with gentle rocking or agitation.

Detection

VerdiBlot supports multiple detection modalities:

- chemiluminescent (ECL)
- fluorescent (280–800 nm)
- colorimetric

Follow your laboratories standard practices and incubation times for application of primary and secondary antibodies.

For fluorescence, ensure compatible secondary antibodies and imaging system filters are used.

For ECL detection, incubate the membrane with primary and HRP-conjugated secondary antibodies, wash thoroughly, and apply substrate immediately before imaging.

Tips for all detection methods:

- Select the method best suited to your target and equipment.
- Optimize exposure time and settings to maximize signal and minimize background.
- Follow lab-specific protocols for washing and handling to ensure reproducibility.

4- Protein transfer (Semi-dry transfer, including third party equipment for fast semi-dry transfer systems)

VerdiBlot membrane is compatible with fast semi-dry protein transfer systems (e.g: Trans-Blot™ Turbo Transfer System from BioRad)

Buffer	Modified Towbin buffer (**semi-dry transfer**): 100 mM Tris, 190 mM glycine, 20% (v/v) MeOH or EtOH. Also compatible with proprietary transfer buffers from BioRad and Millipore.
Transfer sandwich setup	Follow instructions of the manufacturer. A simple transfer sandwich can be assembled from bottom to top in the following layers **Bottom filter paper> VerdiBlot membrane> Gel> Top filter paper.** Follow recommendations for wet transfer found in this Guide.

For semi-dry transfers, confirm that the electrode plates are clean and that the membrane remains in tight contact with the gel throughout the process.

Transfer protocol compatible with Trans-Blot™ Turbo Transfer System from BioRad

System	Target protein	Current/Voltage	Transfer Time
Semi-Dry Transfer	High MW	0.8 A constant, 14 V (1 gel)	20-30 min
	Mixed MW	0.8 A constant, 20 V (1 gel) / 2.0 A, 20 V (2 gels)	7 min
	Low MW	0.8 A constant, 20 V (1 gel) / 2.0 A, 20 V (2 gels)	5 min

Note 1: When first starting a new project it is recommended to try the High MW conditions, this will produce the best protein transfer. This can later be optimized by switching to Mixed MW settings.

Note 2: for high molecular weight proteins semi-dry and dry transfer methods are not ideal. The selected conditions are meant as an optimization but it will always be imperfect. Whenever possible, select a wet transfer if your focus is high molecular weight proteins.

5- Stripping and reprobing

VerdiBlot membranes are compatible with commonly used stripping buffers and can undergo multiple rounds of stripping and reprobing while retaining target proteins. The goal of membrane stripping is to remove primary and secondary antibodies from a Western blot membrane while leaving the immobilized proteins intact, allowing the membrane to be re-probed with different antibodies. In practice, stripping often results in some loss of immobilized proteins, with the extent of protein loss depending on the stripping conditions, the properties of the target protein and the properties of the membrane. Use only for chemiluminescence and fluorescence detection. For colorimetric detection stripping is not possible as it permanently colors the membrane.

⚠ For VerdiBlot do not exceed temperature of 37 °C for prolonged time during your stripping protocol.

Planning your stripping and re-probing strategy

To design a successful stripping workflow, it is important to plan the order in which target proteins will be probed, stripped, and re-probed. Antibodies with weaker affinity for their target proteins should generally be stripped first, as milder stripping conditions may be sufficient for their removal (e.g., antibodies targeting phosphorylated epitopes may require less stringent stripping conditions compared with antibodies recognizing multiple epitopes). In parallel, antibodies targeting smaller proteins should be prioritized for earlier stripping, as smaller proteins may be more susceptible to loss from the membrane during prolonged or repeated stripping procedures. After each stripping attempt it is necessary to assess its effect which requires first to re-block the membrane and then incubate with secondary antibody only and develop the membrane. If there is still some remaining signal you can either decide to prolong stripping time under the same conditions or to increase its stringency (See table below).

Steps	Mild	Medium	Strong	Strong
1. Stripping	0.1 M glycine (pH 2.2) 0.1 % SDS 1 % Tween-20	0.1 M glycine (pH 2.2) 2 % SDS 1 % Tween-20	Restore™ PLUS Western Blot Stripping Buffer (Thermo Fisher Scientific)	Guanidine hydrochloride-based stripping
	10 – 15 min	10 – 15 min	10 – 15 min	----
	Room temperature	Room temperature	Room temperature	----
2. Washing	Thoroughly remove the stripping buffer by washing 3–5 times with TBS or PBS			
3. Reblocking	Re-block for 20 min using the same blocking buffer as used for the initial blocking step			
4.Control	Incubate with the secondary antibody alone for 1 hour. Develop the membrane, and if signal remains, choose either to prolong the current stripping procedure or to increase its stringency by going back to Step 1.			
5. Reprobing	If Step 4 yields no signal the stripping was successful, proceed to wash off the secondary antibody and reprobe your membrane as you would do after protein transfer (primary and secondary).			

6- Troubleshooting

Issue	Possible Cause	Solution
Weak signal	Insufficient transfer time or uneven membrane activation	Extend or decrease transfer time based on MW of your protein (for low MW proteins – shorter transfer time, for high MW longer transfer time is recommended. Make sure the membrane is properly wetted in the transfer buffer.
Membrane overheating or melting	Excessive current	Use cooling block or pre-chilled buffer for wet transfer. For fast semi-dry transfer systems reduce the current and carefully follow the instructions in section 4 of this Guide.
Uneven transfer or spots	Air bubbles or poor contact	Roll out bubbles, ensure uniform stack pressure
High background	Incomplete blocking or overexposed	Optimize blocking time, blocking agent and antibody dilution, or exposition time
Loss of high MW proteins	Short transfer time	Increase transfer duration or reduce methanol concentration. When extending the transfer time - make sure the system is not overheating
Target current not reached in semi-dry transfer	Insufficient buffer in the transfer sandwich	Ensure that the sandwich transfer is well soaked. If current still does not reach the target, add a small additional volume of buffer (10-50ml) over the pre-soaked transfer sandwich.
Floating membrane	Insufficient alcohol in buffer causes uneven wetting	Dip membrane (for 5 sec) in methanol to improve wetting and then equilibrate for few minutes in the transfer buffer

7- Recommended Buffers

- Towbin Buffer (Wet Transfer): 25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3
- Semi-Dry Transfer Buffer: 100 mM Tris, 190 mM glycine, 20% MeOH/EtOH
- TBS-T Wash Buffer: 20 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 7.6
- Blocking Solution: 1-5% milk in TBS-T or 3% BSA for phosphoproteins
- Stripping Buffer: 0.1 M glycine, 0.1% SDS, 1% Tween-20, pH 2.2

For additional technical resources, visit: www.verdiblot.com