User Application Note

VerdiBlot

VerdiBlot - A Next-Generation Membrane Enhancing Western Blot Clarity and Sensitivity in Wide Range of mitochondrial membrane Proteins

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Abstract

Western blotting is a commonly employed technique in numerous laboratories for protein analysis. However, existing membranes can sometimes hinder the detection of specific proteins, often due to inefficient transfer, weak signals, or elevated background noise. To overcome these limitations, VerdiBlot—a next-generation membrane—was developed. This application note highlights its improved performance over conventional PVDF membranes, showcasing better protein retention, reduced background, and clearer resolution of faint bands.

Introduction

Mitochondrial membrane proteins play crucial roles in energy production, apoptosis, and metabolite transport, making their detection by western blot essential for mitochondrial research. However, their hydrophobic nature often leads to poor solubilization and inefficient transfer during blotting. Additionally, high lipid content in mitochondrial preparations can interfere with antibody binding and band clarity. PVDF and nitrocellulose membranes are commonly utilized in Western blotting; however, they often exhibit drawbacks including elevated background signals and inefficient transfer of low-abundance proteins. VerdiBlot represents a novel membrane technology, specifically designed to improve protein binding capacity and signal-to-noise ratio, while maintaining user-friendly handling characteristics.

Objective

To evaluate the performance of VerdiBlot Transfer Membrane (0,3 µm) with a classic wet transfer Western blot in the study of mitochondrial membrane proteins with a MW range of 16-100kDa. The results will be compared directly with a PVDF Transfer Membrane (0,45 µm). The results focus on transfer efficiency, background signal and sensitivity for detection of wide range proteins.

Materials and Methods

Sample preparation

Hek293T cells were seeded in a 10 cm plate at 1000 hours a day before the experiment. The next day, 24 hours later, cells were washed twice with 5 mL ice-cold PBS.

Afterwards, 1 mL of KPBS was added and cells were scraped using a cell scraper. This was followed by a short spin (1000g for 2 minutes at 4℃) to pellet the cells. The pellet was resuspended again in 1 mL KPBS and 25 uL was transferred to a new tube and 2X DDM Lysis buffer was added and was centrifuged at 16000g for 20 minutes at 4℃. The supernatant was transferred to a new tube labelled as **WC**. To **WC**, 16 µL of 4X sample buffer was added. The remaining cells were passed 15 times through a 27G syringe to break the cells. This part was centrifuged at 500g for 5 minutes at 4℃ to pellet the broken cells. The supernatant was transferred to a new tube and centrifuged at 10000g for 10 minutes. From the supernatant, 50 uL of supernatant was taken and 16 uL of 4X sample buffer was added. This was labelled at **Cyto**. The pellet was resuspended using 200 uL of 0.5% DDM lysis buffer and was incubated on ice for 15 minutes. Then it was centrifuged at 14000g for 5 minutes at 4℃. The supernatant was collected and 66 uL of 4X sample buffer was added. This was labelled as **MP**. The protein was quantified using BCA protein assay (Thermo #cat. 23225) and 5 µg, 10 µg and 15 µg of protein were loaded.

Western blotting

Novex™ Tris-Glycine Mini Protein Gels, 4–20%, 1.0 mm, WedgeWell™ format gel (Thermo #cat. XP04202BOX) was used for western blotting. Gel was run at 140V for 120 minutes. Transfer was performed at 45 V for 120 minutes and 100 minutes for PVDF Transfer Membrane, 0,45 µm (ThermoFisher) and VerdiBlot Transfer Membrane, 0,3 µm (LAM-X) respectively. After the transfer, the membranes were blocked in 5% skim milk in TBS-T (Tween 0.1 % v/v) for 1 hour at room temperature. Membranes were incubated with primary antibodies overnight at 4℃. Secondary antibodies conjugated with HRP were used at a dilution of 1:5000 for 1 hour at room temperature. Detection of the specific signal was achieved by Clarity ECL Western Blotting Substrates (Biorad #cat. 1705060S) and images were taken using IQ800 system.

Results

Table 1: *Comparison of PVDF vs VerdiBlot in normal (wet) Western blot transfer.*

Parameter	VerdiBlot	PVDF Standard
Compatibility with Gel	*Compatible*	*Compatible*
Methanol Activation Required	*Not required*	*Required*
Protein Transfer Efficiency	*High*	*High*
Background Signal	*Low*	*Noticeable*
Signal Intensity	*High*	*Medium*
Sharpness of the bands	*Excellent*	*Excellent*
Handling and Durability	*Strong*	*Medium*

Figure 1: Comparative analysis of VerdiBlot and PVDF membranes in Western blotting*. Lysates from HEK293 cells were loaded in different amounts (5-15 µg). WC – first supernatant, Cyto – second supernatant, MP –pellet. SDS-PAGE electrophoresis and wet transfer were performed as described in material and methods. Specific antibodies were used for detection of ASCT2 (70 kDa) and MCT1 (48 kDa) proteins. The protein name is listed on the left along with the size.*

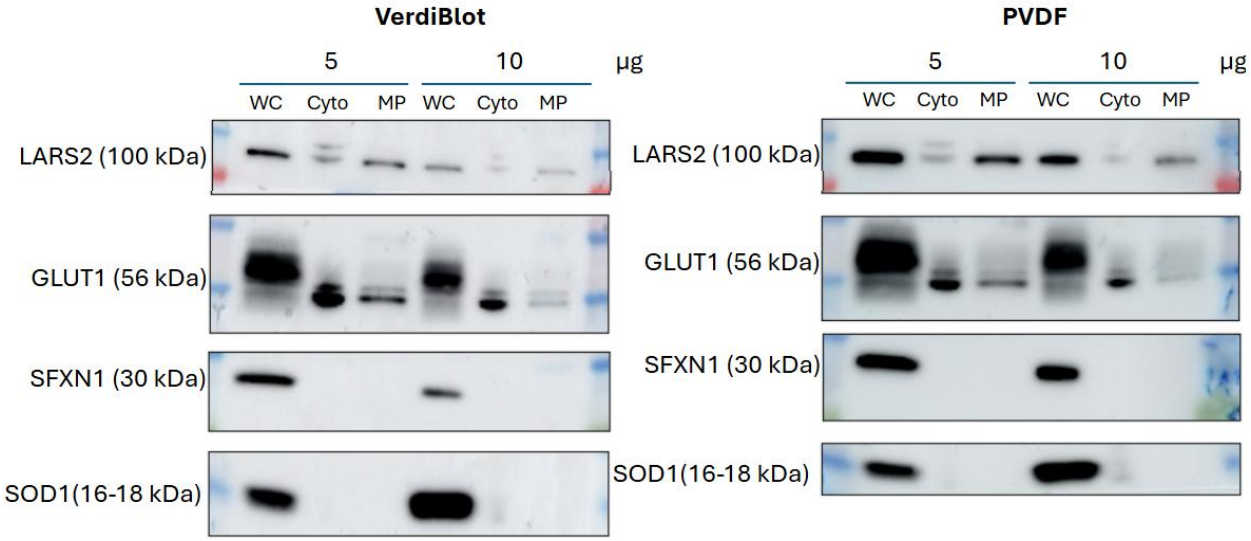

Figure 2: Comparative analysis of VerdiBlot and PVDF membranes in Western blotting – screening of different protein type and size (16 kDa – 100 kDa). *Lysates from HEK293 cells were loaded in different amounts (5 and 10 µg). SDS-PAGE electrophoresis and wet transfer were performed as described in material and methods. The protein name is listed on the left along with the size*

Discussion

VerdiBlot showed comparable signal clarity to standard PVDF membranes, with well-defined bands across a similar dynamic range (Figure 1). Faint bands were clearly detectable, which can be useful when working with low-abundance proteins or antibodies with lower binding capacity. Handling of the VerdiBlot membrane was easier than PVDF, as it is more durable and less prone to tearing during processing. The workflow is also slightly simplified since VerdiBlot does not require activation in ethanol prior to use. As demonstrated in Figure 2, VerdiBlot supports detection of a broad range of protein sizes (16–100 kDa), indicating its suitability for general Western

blotting applications.

Conclusion

- VerdiBlot showed signal clarity comparable to standard PVDF membranes.
- Faint bands were detectable, supporting use with low-abundance proteins or low-affinity antibodies.
- Easier handling due to improved durability.
- No ethanol activation required, simplifying workflow.
- Suitable for proteins ranging from 16–100 kDa

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