LAM+

User Application Note

VerdiBlot

VerdiBlot Transfer Membrane in Western Blot and LC-MS: Practical Workflow and Performance Assessment

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Abstract

Western blotting is a widely adopted technique for protein analysis. The performance of the membrane used during protein transfer has a major impact on signal quality, band clarity, and overall workflow efficiency. Conventional PVDF membranes often require methanol activation and may struggle with issues such as background interference or limited compatibility with certain gel types. The VerdiBlot Transfer Membrane was evaluated as a convenient alternative, offering compatibility with the Bio-Rad Stain Free 4–20% gels that allow rapid UV-based protein visualization. This application note highlights the ease of use, robust immunodetection results, and seamless downstream compatibility of VerdiBlot membranes with LC-MS workflows—all without the need for methanol activation.

Introduction

Mass spectrometry (MS) in combination with western blotting provides a powerful analytical workflow for protein identification, quantification, and post-translational modification analysis. PVDF and nitrocellulose membranes are widely used in Western blotting, however they often present challenges such as additional preparation steps and strong background noise. When preparing samples for mass spectrometry (MS) analysis, selecting the right membrane becomes especially critical. An optimal membrane should combine high protein-binding capacity, chemical compatibility with MS workflows, and robust mechanical stability. This ensures efficient protein recovery, cleaner peptide elution, and minimal contamination, all of which directly enhance MS sensitivity and overall data quality. VerdiBlot Transfer Membrane offers a user-friendly alternative—no methanol activation needed and works smoothly with Bio-Rad Stain Free 4–20% gels, chemiluminescent detection, and LC-MS workflows. It provides a practical option for modern protein analysis without complicating the process

Objective

This application note aims to evaluate the performance of the VerdiBlot Transfer Membrane using a semi-dry Western blot system. Key goals include assessing its ease of use, compatibility with Bio-Rad Stain Free 4–20% gels, suitability for chemiluminescent detection, and effectiveness in downstream

LC-MS.

Materials and Methods

Homogenization

Mouse liver lobe was weighted (196 mg) and placed in 2 mL test tube with 980 µL of RIPA buffer (5 µL per 1 mg of tissue), 10 µL of protease inhibitors (Merck, #P8340) and 100 mg of 1-2 mm glass beads. Sample was homogenized in Precellys 24 device with settings: 6800 oscillation per minute for 30 seconds, 3 times repeated. Homogenate was incubated on ice for 40 minutes and centrifuged for 20 minutes at 14.000 x g/4°C. Supernatant was separated and aliquoted.

Total Protein Assay

Total protein concentration was estimated using standard BCA assay with BSA as a standard protein. The concentration of proteins in mouse liver lysate was 20.7 µg/µL.

1D Electrophoresis and Western Blot

For the electrophoresis 12-well Bio-Rad Stain Free gel 4-20% was used. Each sample (except of standard) was mixed with 5 µL of 6X Laemmlli sample buffer and 1 µL of 2-merkaptoethanol (BME) and heated to 90°C for 10 minutes. Western C Standard (Bio-Rad, #1610376) contains Strep-tagged recombinant proteins (10–250 kD), including three pink reference bands (25, 50, 75 kD). Each well was loaded with a different concentration of mouse liver lysate—ranging from 5 to 100 µg of total protein—mixed with 5 µL of 6X Laemmli sample buffer and 1 µL of β-mercaptoethanol (BME); Western C Standards (5 µL) were loaded into wells 1 and 7 as molecular weight markers. The gel was mounted to electrophoretic device filled with tris-glycine running buffer, connected to electric source (U=200 V, I=80mA) and run until bromphenol blue reached the end of the gel. Gel was scanned in ChemiDoc scanner with Stain Free settings.

Western Blot Transfer

Gel was cut into two halves (between well 6 and 7). Left half was blotted with Trans-Blot Turbo Mini 0.2 µm PVDF Transfer Pack (Bio-Rad, #1704156), and right half was blotted with VerdiBlot Trasnfer Membrane 0,3 µm (LAM-X) in self-assembled sandwich.

Western Blot was performed in Trans-Blot® Turbo™ Transfer System (Rio-Rad, #1704150) with Mixed MW settings (U=25 V, I=1.3 A, t=7 minutes) with both halves of gel.

Immunodetection

Bio-Rad and Lam-X membranes were washed twice (50 mL TTBS/5 minutes) immediately after blotting and blocked with 1% casein/PBS (Bio-Rad, #1610782) for 90 minutes at room temperature. Membranes were washed three times (50 mL TTBS/5 minutes) after blocking and laid down onto parafilm sheet and covered with mixture of antibodies against histone H1 (Merck, #SAB4501366, 1:1000) and GADPH (Merck, #G9545, 1:2000). Antibodies were diluted with TTBS. Membranes were incubated overnight at 4°C in Petri dish with paper wicks wetted with water to ensure air moisture over membranes to prevent evaporation of antibodies solution. Next day membranes were washed three times (50 mL TTBS/5 minutes) and transfered onto new parafilm sheet and covered with mixture of secondary antibodies Immun-Star Goat Anti-Rabbit (GAR)-HRP Conjugate (Bio-Rad, #1705046, 1:2000) and Precision Protein StrepTactin-

HRP Conjugate, (Bio-Rad, #1610381, 1:6000). Antibodies were diluted with TTBS. Streptactin -HRP Conjugate is added to visualize protein standard ladder. Membranes were incubated 2.5 hours at room temperature. Membranes were washed three times (50 mL TTBS/5 minutes) after incubation with secondary antibodies and visualized using Clarity Western ECL Kit (Bio-Rad, #1705060) by the recomendation of provider. Membranes were scanned in ChemiDoc scanner with ChemiHigh settings.

Identification of Bands with LC-MS

VerdiBlot membrane was washed twice with 100 mL of UHQ water and stained with Colloidal Gold Total Protein Stain (Bio-Rad, #1706527) for 30 minutes. Picked bands were cut out using scalpel from membrane and washed twice with 100 mM ammonium bicarbonate (ABC). Bands were incubated with 100 mM BME/100mM ABC for 30 minutes at 37°C. Reduction solution was discarded, and proteins were alkylated using 100mM CAA (2-chloroacet-amide)/100mM ABC for 30 minutes at room temperature in dark. Alkylation solution was discarded, and bands were immersed in 100 µL of 100mM ABC and 10 µL of trypsin (Merck, #TRYPSEQ-RO, 50 ng/µL in 1mM HCl) and incubated overnight at 37°C. Digestion was stopped by addition of 10 µL neat formic acid. Pieces of membrane were discarded, and peptide solutions were evaporated to dryness on SpeedVac. Peptides were reconstituted in 25 µL of 5% ACN/0.1% FA and filtered through 0.22µm PVDF filter directly to LC vials. LC-MS analysis was performed on nanoElute-timsTOF Pro (Bruker), 25 cm x 75 µm PepSep-Ultra column, 60 minutes gradient 0-35% ACN/0.1%FA. Raw files were processed in MS Fragger software with standard identification settings against Mus musculus Uniprot database. Contaminants (keratin, trypsin, etc.) and proteins with a probability lower than 0.99 and identified by fewer than two peptides were removed from the resulting list of proteins.

Results

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

Parameter	VerdiBlot	PVDF Standard
Compatibility with Stain Free Gels	*Compatible*	*Compatible*
Methanol Activation Required	*Not required*	*Required*
LC-MS Compatibility	*Compatible*	*Compatible*
Protein Transfer Efficiency	*moderate*	*High*
Background Signal	*Very low*	*Noticeable*
Signal Intensity	*High*	*Very high*
Sharpness of the bands	*Excellent*	*Excellent*

Parameter	VerdiBlot	PVDF Standard
Handling and Durability	*Strong*	*Strong*

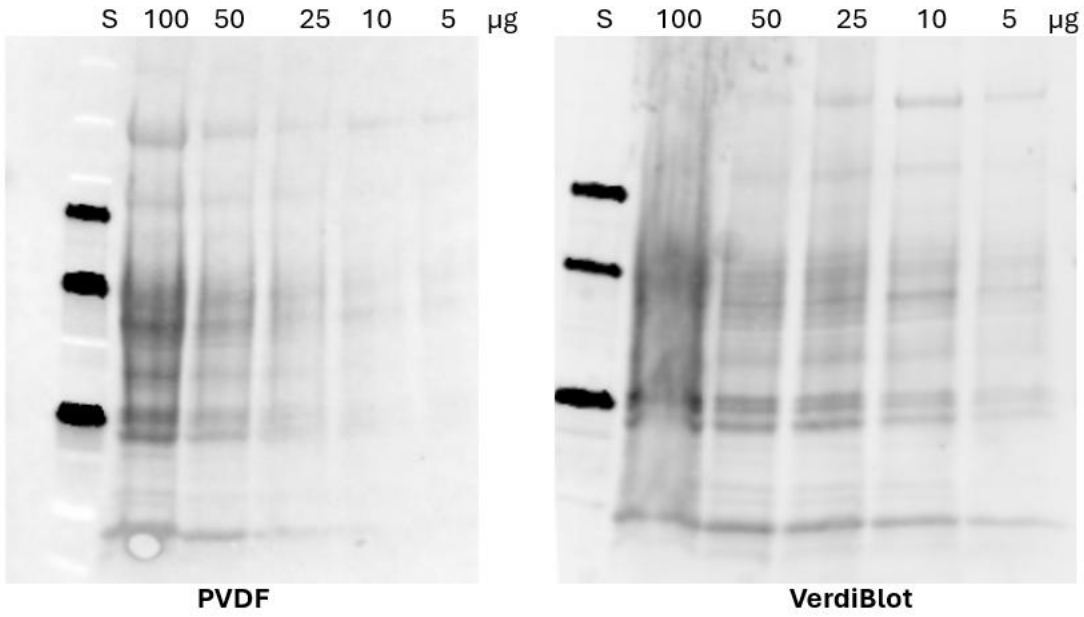

Figure 1: Comparative analysis of PVDF and VerdiBlot membranes in Western blotting – total protein visualization after western blot transfer*. Mouse liver lysate was loaded in different amounts (5-100 µg) and Western C Standards labeled as S. SDS-PAGE electrophoresis and wet transfer were performed as described in material and methods.*

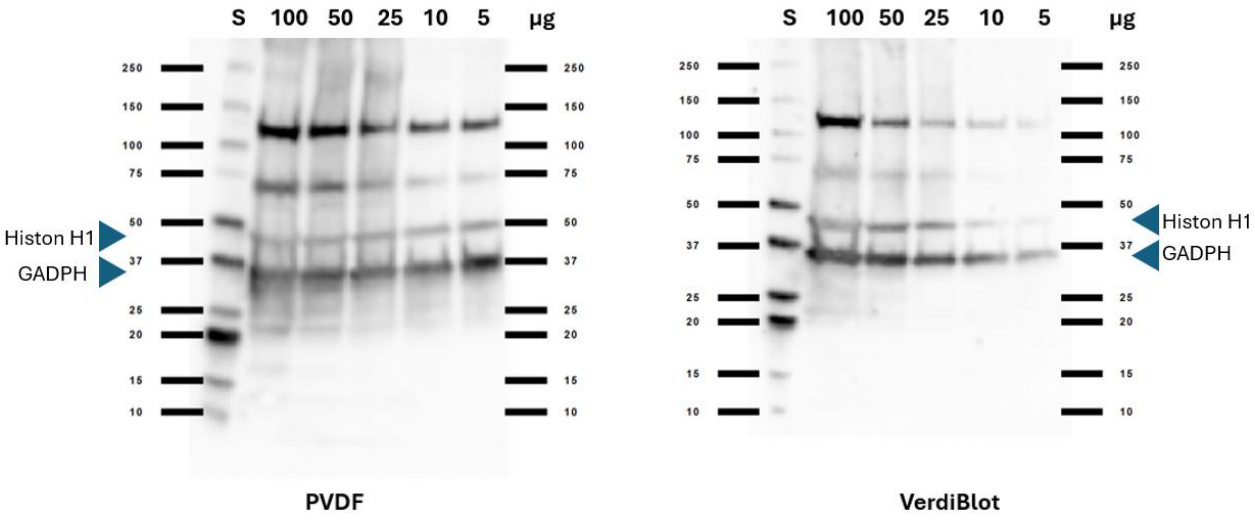

Figure 2: Comparative analysis of PVDF and VerdiBlot membranes in Western blotting*. Mouse liver lysate was loaded in different amounts (5-100 µg) and Western C Standards labeled as S. SDS-PAGE electrophoresis and wet transfer were performed as described in material and methods. Detection of GAPDH (34 kDa) and histone H1 protein (44 kDa).*

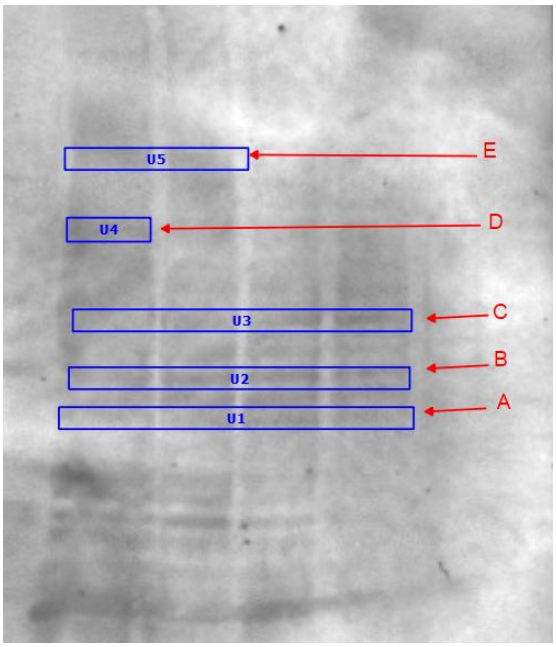

Figure 3: *VerdiBlot membrane stained with Colloidal Gold Total Protein Stain. The most visible band C (GAPDH) was used for the LC-MS.*

Discussion

The VerdiBlot membrane was evaluated in a comparative Western blotting workflow using mouse liver lysate. Each well was loaded with decreasing concentrations of total protein (100 µg to 5 µg), and the gel was split for simultaneous blotting onto VerdiBlot (LAM-X) and a standard PVDF membrane (Bio-Rad). VerdiBlot membrane examinate clear immunodetection of both GAPDH (34 kDa) and histone H1 protein (44 kDa) with all relevant bands resolved across the dilution series (Figure 2). Visualization was performed via chemiluminescence, with the VerdiBlot membrane producing comparable band sharpness and minimal background when using standard detection antibodies. Importantly, VerdiBlot Transfer Membranes proved fully compatible with UV detection of total protein content based on the Stain Free gel after Western Blot transfer (Figure 1). This enabled quick assessment of protein load across samples and validated transfer efficiency.

Following blotting, VerdiBlot Transfer Membranes were also subjected to LC-MS based protein identification. After colloidal gold staining and manual excision, bands of interest were digested and analyzed via LC-MS. Dozens of proteins were confidently identified, confirming that VerdiBlot membranes are well-suited for proteomic workflows without requiring changes to standard digestion protocols.

In addition, VerdiBlot Transfer Membranes required no pre-activation with methanol, simplifying the blotting process and reducing variation. Overall, VerdiBlot offered a streamlined, sensitive, and versatile solution for both immunodetection and downstream mass spectrometry.

Conclusion

- VerdiBlot Transfer Membrane offers a methanol-free, user-friendly alternative to traditional PVDF membranes.
- Compatible with Bio-Rad Stain Free 4–20% gels, enabling UV-based protein visualization pre-transfer.
- Delivers clear, low background immunodetection of key protein targets like GAPDH and histone H1.
- Seamlessly supports downstream LC-MS protein identification after colloidal gold staining.
- Simplifies Western blot workflows while maintaining robust analytical performance.

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