Western Blot with Vacuum Blotter and ECL Detection by KETA CLX

INTRODUCTION

The **Western blot** method, which is widely used in research to separate and identify proteins, is based on protein interactions with detection probes. Proteins are traditionally separated on an electrophoresis gel before being transferred to a membrane. The membrane is then typically treated with antibodies that are specific to the protein of interest. For detection methods, a number of detection techniques, including colorimetric, radioactive, chemiluminescent, as well as fluorescent.

Protein transfer is now easier than ever with the **Smart Blotter**, as opposed to the old technique. The protein sample is vacuum-transferred to the membrane instead of separated by gel electrophoresis, which significantly reduces the experiment time. Transfection directly into the membrane can enhance each sample's signal and make detection simpler. Smart Blotter, on the other hand, enables the use of different probes, such as nucleic acids (Southern and Northern Blot) or monoclonal antibodies (Western Blot), which are based on varied membranes and target samples. While Smart Blotter can examine a large number of samples (up to 48 at a time), it does not provide information on the molecular weight or modification of the specific target samples. To get good results, the material of the membranes, sample volume, antibodies, vacuum pressure, suction flow rate, techniques, and so on must be optimized before to analyzing the samples. This article demonstrates the differences in transfer between PVDF and NC membranes, allowing users to gain a full picture and make the best decision for their researches.

The protein signal is detected using the **KETA CLX** Chemiluminescent system. Enhanced chemiluminescence (ECL) is the most commonly used sensitive detection technique in Western blot analysis. The basic idea behind this method is that light is emitted during the chemical process of oxidizing a substrate with hydrogen peroxide. KETA CLX is ideal for detecting chemiluminescent signals in Western blots, with a detection limit of 2.4 pg pure transferrin. The images are captured using the “Auto Capture” option in order to achieve qualitative images.

MATERIAL

- Transferrin (HOLO), Human Plasma (BioVision, US)
- Transferrin Antibody (BioVision, US)
- AffiniPure Goat Anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, US)
- Chemi-Lumi One L (Nacalai Tesque, Japan)
- Bradford reagent (Sigma Aldrich, US)
- Methanol anhydrous (Macron Fine Chemicals, US)
- 1X 0.1% TBS-T
- 5% milk/TBST blocking buffer
- Phosphate Buffered Saline
- Double-distilled water or deionized water
- 7 x 8 cm filter paper
- 7.5 x 8.5 cm Immobilon-P PVDF Membrane 0.45 µm pore size (Sigma Aldrich, US)
- Instruments: SpectroArt 200S, Smart Blotter SB-10, and KETA CLX (Wealtec)

METHODS

Determine protein concentration

- Dissolve 5 mg Transferrin (HOLO), Human Plasma in 500 µL PBS.
- Dilute 100 µL Human Transferrin in 900 µL PBS (0.1X)
- Determine protein concentration by Bradford assay:

  Blank: 50 µL PBS in 1500 µL Bradford reagent

  Sample: 50 µL 0.1X solution in 1500 µL Bradford reagent
- Incubate for 10 min at room temperature in the dark before analysing with the SpectroArt 200S.

Rapid Western Blot with Smart Blotter

- For Western Blot, 0.05691 µg/µL of protein concentration is used.
- Soak 2 pieces of filter paper in PBS for 5 sec.
- Place two pieces filter paper on the support plate, making sure there are no bubbles on it.
- Soak the PVDF membrane in methanol for 5 sec, DDW for 2 min, and PBS for 5 min. Only soak the NC membrane in PBS solution.
- Place membrane onto the filter paper. Then close the top block.
- Insert the plug and lock the flip stoppers on both sides while pressing the top block.

- Connect vacuum tube to the tap, as well as the Smart blotter.
- Fill each well with 200 µL of protein sample.
- Turn on the valve and wait until there is no more liquid in the well.
- Add 200 µL of PBS into each well and open the valve until the buffer pass through the membrane. Repeat process one more time for a second wash.
- After finishing, take the membrane out and reactivate it with methanol for 5 sec.
- Shake membrane for 1 h with 25 mL of 5 % milk/TBST blocking buffer.
- Discard the blocking buffer and wash three times with TBST.
- Shake membrane for 1 h with 1:15000 primary antibody
- Wash three times with TBST for 10 minutes each time.
- Shake membrane for 1 h at 60 rpm with 1:50000 secondary antibody
- Wash three times with TBST for 10 minutes each time.
- Keep the membrane in DDW for ECL.

ECL detection

- Let the ECL reagents cool down to RT. Prepare 1400 µL mixture of luminol solution and peroxide solution in a 1:1 ratio.
- Spread the reagent mixture to cover all membrane. Wait a minute for reaction, then observe the results by KETA CLX.

RESULT

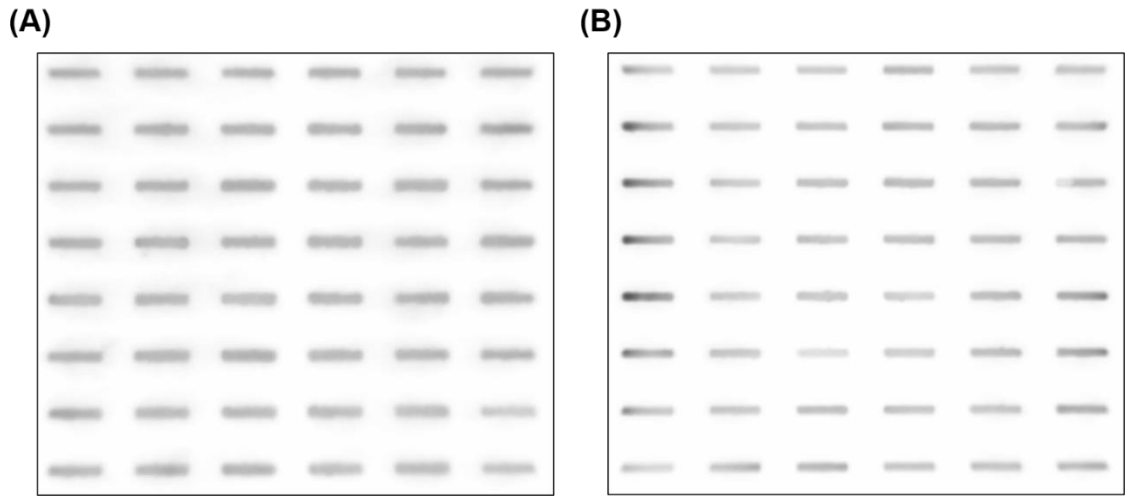

Figure 1. Smart Blotter Western blot with (A) PVDF and (B) NC membranes. The images were captured using the KETA CLX in Auto Capture mode.

DISCUSSION

Wealtec Smart Blotter is the rapid and simply method for Western blotting. PVDF and NC membranes with a Human Transferrin concentration of 0.05691 μg/μL were employed in this work. They are presented with ECL chemiluminescence reagent, and detected by KETA CLX Chemiluminescent system.

As shown in Figure 1, the form of the slots on the NC membrane is more shaped than the pattern of the slots on the PVDF membrane. However, when the transfer uniformity was compared particularly on each slot, the PVDF membrane performed better. The distinction might result in differences in the properties of both membranes, such as restrictions on solvent tolerance and binding capacity. So, users should clarify them before any chose to conduct experiments.

CONCLUSSION

Because the sample can be transferred directly onto the membrane with the Wealtec Smart Blotter, the signal is strengthened and detection is simplified. Owing to the Smart Blotter's design, which includes an easy-to-assemble quick-fit connection and a screw-free flip stopper, up to 48 samples would be transferred at once, making it ideal for large screening. It may be used with a variety of probes and membranes, but users should understand vacuum pressure, suction flow rate, methods, and the properties of the materials that they intend to employ.

Wealtec KETA CLX is recommended for high-sensitivity chemiluminescence detection, having a detection limit of 2.4 pg pure transferrin. With the most optimal interface, customers could simply obtain high-quality image results and create their own report style.

REFERENCE

- Mahmood, T., & Yang, P. C. (2012). Western blot: technique, theory, and trouble shooting. *North American journal of medical sciences*, *4*(9), 429.
- MacPhee, D. J. (2010). Methodological considerations for improving Western blot analysis. *Journal of pharmacological and toxicological methods*, *61*(2), 171-177.

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