Western Blot by using Wealtec Smart Blotter

INTRODUCTION

The **Western Blotting** (refer to as immunoblot) was one of the protein analysis techniques introduced in 1979. It was based on the interaction between protein and detection probes (normally antibody). First, separate the protein mixture with their molecular weight, isoelectric point, or net electric charges by using one or two dimension SDS-PAGE or native PAGE. And then transfer the separated protein to the membrane (usually nitrocellulose or PVDF membrane). Treat the membrane with labeled probes to locate the target sample. After that, analyze the result with different detection categories such as colorimetric, radioactive, chemiluminescent, or fluorescent detections.

Smart Blotter for dot blot assay is a simplest and rapidest method for analyzing samples. Smart Blotter is used to transfer the samples directly to the membrane by vacuum without the separation by gel electrophoresis. Moreover, the focusing effect on the membrane can also intensify the signal of each sample solution and make the detection more easily. In addition, with different membranes and different target samples, users can apply Smart Blotter system to different kinds of probes, such as nucleic acids (Southern and Northern Blot) or monoclonal antibodies (Western Blot). However, this method did not provide the information about the molecular weight or the modification of the specific target samples. When there have large amount of samples that needed to be analyzed, Smart Blotter can provide preliminary screen up to 48 different samples at one time and significantly shorten the experiment time.

As mentioned above, **Smart Blotter** for dot blot assay is the simplest process to analyze huge amount of samples, there still have a lot of factors that affect the blotting result. Major factors are the material of membranes, vacuum pressure, suction flow rate, sample amount, antibodies, presentation methods, and etc. All these factors are needed to be optimized before screening the samples. In this article, it provides an optimized condition for the cell lysate target samples that might be useful for customers to design their experiment.

MATERIAL

- 293T cell lysate (Emo Biomedicine Corp., Taipei, Taiwan)
- ECL reagent: Western Lighting™ Chemiluminescence Reagent Plus.
- 1st Antibody— ß-actin mouse monoclonal IgG antibody (1:500) (Santa Cruz Biotechnology)
- 2nd Antibody— Goat anti-mouse-IgG RLP Ab (1:3000) (Santa Cruz Biotechnology)
- Membranes:
  0.45 µm pore size of PVDF membrane (PolyScreen® Transfer Membrane, PerkinElmer™)
  0.45 µm pore size of Nitrocellulose membrane (Immobilon-NC, Millipore™)
- Smart Blotter system (Wealtec)
- ChemiStage CC-16 mini (Wealtec)

METHODS

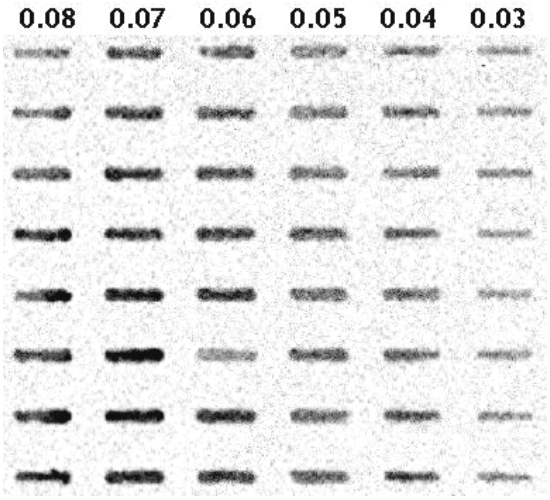
- Prepare series dilutions of the 293T cell lysate.
- Pre-cut the filter paper (7.5 x 9 cm) and membranes (7 x 8.5 cm).
- Soak a piece of the 3 mm filter paper into the transfer buffer.
- Put the filter paper on the support plate and make sure that there have no bubbles on it.
- Pre-treat one or two PVDF (or NC) membrane in the order, which first putted in methanol (or transfer buffer) for 15 seconds, second putted in ddH_2O for 2 minute, and then transfer to PBS buffer for 5 minutes.
- Make sure that the membrane is not dry out before operation.
- Put the membrane on the filter paper, and make sure to cover all the slots on the support plate. Then close the top block.
- Press the top block slightly. Plug in and close the flip stopper on both sides while it is still pressing.
- Adjust the clamp with the diameter of the tube is 3 mm, and clip it on the connecting tube.
- Adjust the water flow rate of the aspirator pump with 20 ~ 100 ml/min.
- Assemble the Smart Blotter system to the aspirator pump.
- Apply 200 µl of PBS buffer to each well. Make sure to apply the sample in the

center of the slot with good care. Avoid forming the bubbles in the bottom of the slots.

- Connect to the aspirator pump and turn on the hydrant to apply the vacuum.
- Once the buffer all pass through the membrane, disconnect the quick connector to break the vacuum. Then connect it again.
- Apply 200 µl of different concentrations of 293T cell lysate solutions to each well.
- Turn on the hydrant to apply the vacuum again.
- Once the solution all pass through the membrane, disconnect the quick connector to break the vacuum.
- Disconnect the module and dry the membranes to have the slot turn into white color on the filter paper.
- After dry the membrane, place the membrane into a 5 % milk/TBST blocking solution and incubate it for 30 minutes.
- Discard the blocking solution and replace with 1^{st} antibody solution (1:1000, dissolved in 5 % milk/TBST) solution. Incubate for 1 hour at room temperature with agitation.
- Wash the membrane with TBST for three times and 10 minutes for each.
- Incubate with 2^{nd} antibody solution (1:3000, dissolved in 5 % milk/TBST) for 1 hour in RT with agitation.
- Wash the membrane with TBST for three times and 10 minutes for each.
- Pipette 0.5 ml enhanced luminol reagent and 0.5 ml oxidizing solution (1:1) onto the membrane and allowed to react evenly for over than 1 minute.
- Observe the result with ChemiStage CC-16 mini.

Result

(A)



(B)

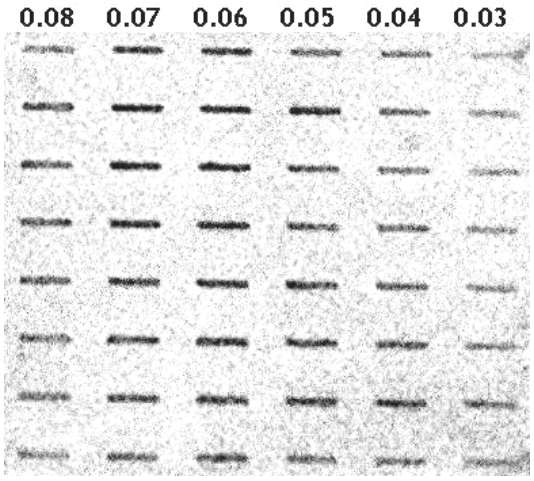
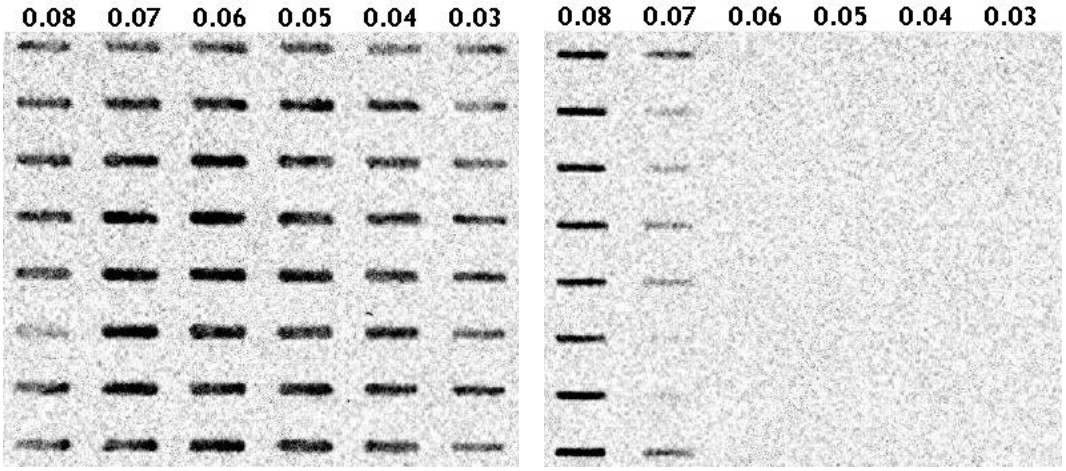


Figure 1. Series diluted samples test of Smart Blotter with one piece of different membranes, (A) for PVDF and (B) for NC membrane. The number on the top of the pictures refers to different protein concentrations from 0.08 to 0.03 mg/ml.

(A)



(B)

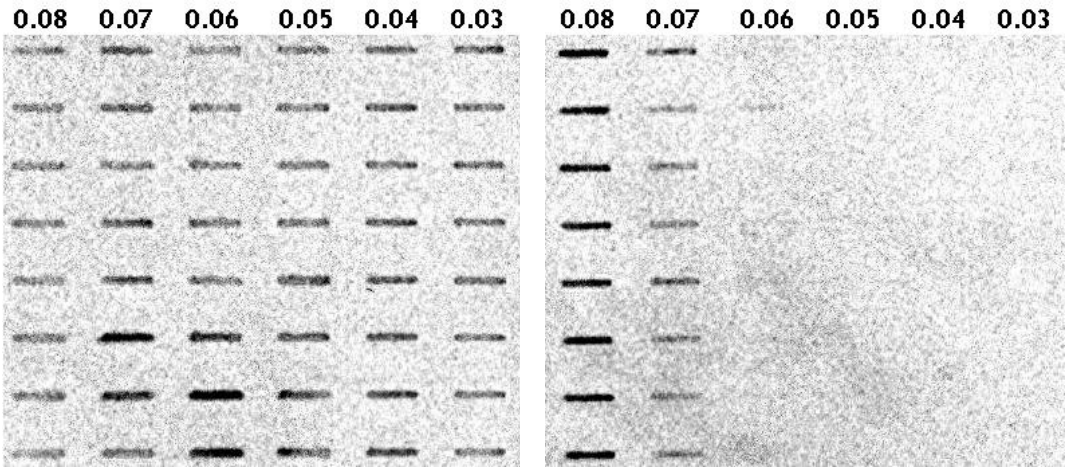


Figure 2. Series diluted samples test of Smart Blotter with different membranes, (A) for PVDF and (B) for NC membrane. The number on the top of the pictures refers to different protein concentrations from 0.08 to 0.03 mg/ml. Membranes on the left side are the top layers of two membranes test and on the right are the bottom layers.

Discussion

As the result showed in figure 1 and figure 2, both PVDF and NC membrane can be used in Western blotting method with the target sample of 293T cell lysate by using Wealtec Smart Blotter module to transfer, presented with ECL chemiluminescence reagent, and detected by ChemiStage CC-16 mini system. Regarding the characteristics differences for both membranes, it is very important to be mentioned that membrane itself has the limitations on solvent tolerance, binding capacity, and mechanical strength. User should read the instruction of the membrane before using it at the very first time.

Comparing the shape of the slots in figure 1 with different membranes, the slots are formed more sharp on NC membrane than on PVDF membrane. According to this feature, NC membrane will be a better option when the sample volume is barely a trace. Besides, according to the manual of the membranes, the capacity of the membrane is 80~100 $\mu g/cm^2$ for NC membrane and 100~200 $\mu g/cm^2$ for PVDF membrane. In the figure 2, the loaded protein amount is about 193 $\mu g/cm^2$ while sample concentration is 0.06 mg/ml with 200 µL sample volume. The slots are observed on the top layer of the membrane while the protein concentration is from 0.08 to 0.03 mg/ml and also observed on the bottom layer while it is higher than 0.07 mg/ml. According to the observation of figure 2, the result indicates it is better to have cell lysate protein concentration under 0.06 mg/ml for PVDF membrane to prevent the waste of samples and also get the best signal.

Furthermore, there have other factors may affect the transfer result, for example, the pressure setting, the suction flow rate of the aspirate pump, the sample volume, and etc. In the former tests, high pressure and fast flow rate setting will make the protein pass through the first membrane and focus on the second one. Higher sample volume would dilute the focusing effect on the membrane. (Data not shown) Therefore, user should adjust possible factors upon various application requirements to optimize the experiment conditions to get the best result.

The experiment condition in this article has been optimized after many times of tests to be suitable for the target sample of 293T cell lysate by using Wealtec Smart Blotter system. The condition we used here can be a reference for users who is the first time to perform the blotting by using Smart Blotter.

REFERENCES

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