Comparison of Immunoblotting Sensitivity between Vacuum Blotter and Traditional Western Blotting

INTRODUCTION

Dot-blotting technique is a high-throughput transfer method to transfer DNA, RNA or protein samples onto membrane for further experience. It helps users to narrow down candidates out of large amount of samples, for instance, antigen diagnosis from a bundle of patient samples. Wealtec's Smart Blotter, a new revolution of dot blotter, utilizes "flip stopper" to replace of annoying screws used in the old system. In addition, it performs transfer with easy-operated aspiratory pump rather than the traditional complicated vacuum pump. Furthermore, the leakage proof slot design allows fast transfer with no contamination between wells. Smart Blotter is suitable for both nucleic acid and protein samples. However, when it is applied to immunoblotting (protein-antibody interaction), some concerns might be raised by users: First, the time cost of transfer procedures in Smart Blotter system is much less than in traditional Western blotting, and the transfer efficiency in Smart Blotter system should be noticed. Second, the protein molecules in Smart Blotter system aren't separated on acrylamide gel prior to stack up in a band-shape area on the membrane. The accumulated molecules might affect the antibody binding ability and mask the emission of chemiluminescence signal from each other. Here in this study, the immunoblotting sensitivity of Smart Blotter was compared with traditional Western blotting.

MATERIALS

- V-GES, E-Blotter, Smart Blotter and KETA ML imaging system (Wealtec)
- Protein sample: Human lung cancer a549 cell lysate
- 1st antibody: anti-actin, 2nd antibody: goat-anti-mouse-IgG-HRP(Santa Cruz)
- Chemiluminescent: ECL Enhanced Chemiluminescence reagent (Millipore)
- PVDF membrane (PerkinElmer)
- BP-C blotting paper

PROCEDURES

1. Protein samples were transferred onto PVDF membranes by traditional Western blotting or Smart Blotter:

   Traditional Western Blot:

   a. Protein samples were separated by 12% SDS-PAGE, and then transferred from gel to PVDF membrane by E-blotter system.

   Smart Blotter:

   a. Membranes were pre-moistened by passing through 200 µl PBS buffer.
   b. Samples were loaded into wells and blotted onto membrane by using aspiratory pump.
   c. Wait for the membrane to dry out.
2. Membranes were hybridized with 1st antibody for an hour and then washed three times with TBST buffer for 10 minutes.
3. Replace the antibody to 2nd antibody and hybridized for another hour.
4. After hybridization, membranes were washed three times with TBST buffer for 10 minutes each.
5. Chemiluminescence signals were presented by ECL and detected in KETA ML imaging system.

RESULT

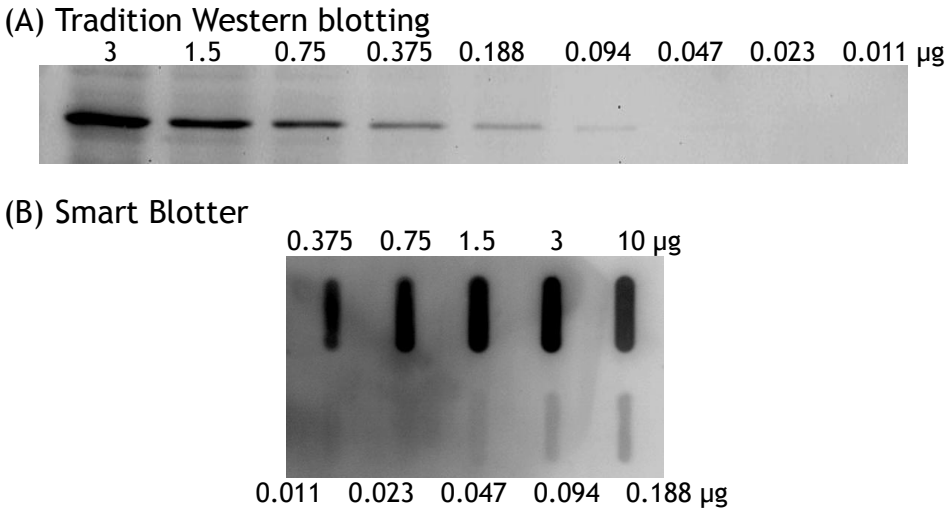

Figure 1. *Chemiluminescence signal detection in KETA ML imaging system and exposure for 400 seconds without turning on the binning function. (A) Traditional Western blotting. (B) Smart blotter.*

DISCUSSION

In the result that is showed above, the immunoblotting sensitivity of Smart Blotter is as well as traditional Western Blotting (46.9 ng / band could be detected), even better. The accumulated molecules do not affect the antibody binding ability or mask the chemiluminescence signal from each other. Furthermore, the transfer efficiency in the Smart Blotter system is better than traditional Western Blotting. About the sample lost issue, using of Smart Blotter to transfer will have lower sample lost during transfer procedures. Besides, it should be noticed that the transferred samples in the Smart Blotter system are not separated by gel electrophoresis and exist with a mixture form. Hence, the specificity of antibody should be confirmed prior to the experiment to prevent the interferences from non-specific binding signals. In conclusion, Smart Blotter is a useful tool with extra convenient operation for fast screening of large amount of samples with high sensitivity immunoblotting.

Yi-Ta, Chen
Product Manager
Yu-Chia, Lin
Application Specialist

Wealtec Bioscience Co. Ltd.
27Fl. No. 29-1 Sec.2, Jungjeng E. Rd., Danshuei Jen, Taipei, Taiwan 25170
TEL: +886-2-8809-8587 FAX: +886-2-8809-8589; http://www.wealtec.com
Email: sales@wealtec.com