

Crystalgen, Inc.

1-800-216-3287

One-Hour Western

Introduction

The western enhancer was designed to help you get your Western blot result quick and easily. It only takes 1-1.5 hour to replace the 4-5 hours or overnight process of the classical western blotting.

Features:

- 1. The rapid process:** the kit was designed to take one hour (1/2 hour for primary, 1/2 hour for secondary antibody binding and 8 min. washing to obtain a desired Western blot data.
- 2. The time saved:** It takes only 1-1.5 hour instead of the 4-5 hours classic western process, with this kit, you can efficiently probe and re-probe membrane for Western, and Dot blots with multiple antibodies or with multiple concentration of an antibody for a desired result.
- 3. The money saving:** One-Hour Western (OHW) is designed to increase the affinity of antibody to target antigen by 6-10 times in comparison to the classical Western method. And the used antibody in OHW antibody binding solution can be kept at 4°C for 4-6 weeks.

For a desired Western Data:

- 1). Please make sure your membrane and anti-body are of good quality.
- 2). If the background is higher than desired, it can be modified by increasing the percentage (2-5%) of Milk (or 1-5% BSA), or decreasing the antibody concentration.
- 3). If a antibody signal is weak, it can be modified by prolonging the exposure or increasing the antibody concentration, or using 0.5% Milk (0.1%BSA) in 1x HHWE as antibody dilution.

Content Table

Catalog No.	Name	Size	Store
300-904-050	One-Hour Western Kit 1 st Antibody Binding Enhancer 10X 2 nd Antibody Binding Enhancer 10X Rapid Wash buffer, 10X	50 ml/bottle x 3	At 4°C for 1 year,

Protocol

Step	Action
Step 1	<ul style="list-style-type: none"> • Diluted the 10X 1st Antibody Binding Enhancer (ABE) with D.W. (H₂O) to be as the 1X solution.. This solution can be kept at 5-29 °C for 6-8 weeks; at 4 °C, 6 months or longer. • Add 2 % milk or 1% BSA to the 1X ABE to be as the ABE 1st antibody blotting solution. This solution can be kept at 4 °C for 4-6 weeks.
Step 2	<ul style="list-style-type: none"> • Put a western blot membrane (transferred or stripped, in the ABE antibody blotting solution with Primary antibody*, then shake at RT for 30 min. • Rinse the membrane with PBS-T** for 20 seconds (shaking by 3-4 times) ** PBS-T= 0.1% tween 20 in 1X PBS. • Wash with 1X Rapid Wash buffer for 2 min. • Wash with PBS-T for 20 seconds. • Place the membrane in 1X 2nd antibody binding enhancer with Secondary antibody, then shake at RT for 30 min. • Wash with the PBS-T for 20 seconds, Repeat one more time. • Wash with 1X Rapid Wash buffer for 5 min. • Wash with PBS-T for 3 min. Repeat this one more time. • Develop with substrate or by ECL, or other accepted methods.
Notes	<ul style="list-style-type: none"> • Not guarantee for a dried used western membrane to have the desired result.

Working Experience

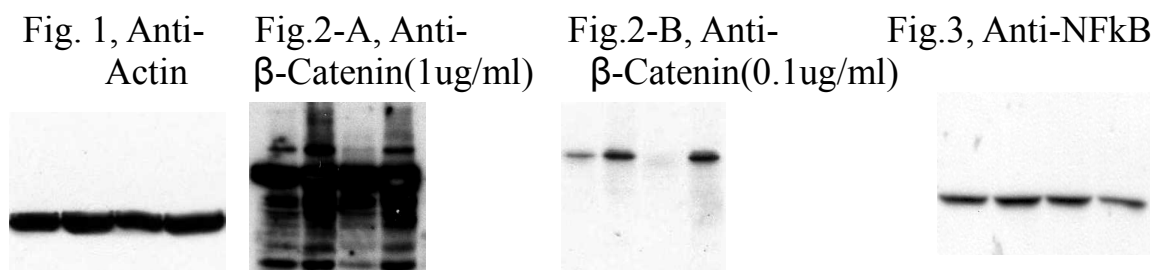


Fig.1, Mouse tissue protein, used the actin 1: 30,000.

Fig.2, **Fig.2-A**, Mouse tissue protein, the antibody –β-Catenin, 1ug/ml, 2 % Milk-HHWE, the result shows the high concentration antibody used.

Fig.2-B, The good result was obtained from the same membrane-**Fig.2 A**, re-probed with Anti- β -catenin (0.1ug/ml) in 2% milk-HHWE

Fig.3 Human cell line protein, antibody-NF κ B p50, E-10 (Santa Cruz, Cat#, sc-8414), 0.66ug/ml in 2% Milk-HHWE.

Reference:

1. Tibes R, et al.: Reverse phase protein array: validation of a novel proteomic technology and utility for analysis of primary leukemia specimens and hematopoietic stem cells. *Mol Cancer Ther.* 2006 Oct; 5(10):2512-21.
2. Golding MC, et al.: Suppression of prion protein in livestock by RNA interference. *Proc Natl Acad Sci U S A.* 2006 Apr 4; 103(14):5285-90.
3. Bleuming SA, et al.: Altered bone morphogenetic protein signaling in the *Helicobacter pylori* infected stomach. *J. Pathol*, 2006 Jun; 7-9(2): 190-7.
4. Deyde V, et al.: Identification of a monoclonal antibody from *Peromyscus maniculatus* (deer mouse) cytomegalovirus (PCMV) which binds to a protein with homology to the human CMV matrix protein HCMV pp71. *J Virol Methods.* 2005 Jan;123(1):9-15.
5. Hardwick JC, et al.: Bone morphogenetic protein 2 is expressed by, and acts upon, mature epithelial cells in the colon. *Gastroenterology.* 2004 Jan;126(1):111-21.