Signosis
Innovative Plate Assay Solutions

Transcription Factor Western Blot Detection Assay Kit

Catalog Number WB-0001 **(For Research Use Only)**

Introduction

Western Blotting has become one of the most widespread techniques for detecting a specific protein in a sample. Although this technique has become standard procedure for many labs, each new antibody requires a great deal of optimization. Signosis Transcription Factor Western Blot assay kits provide a pre-optimized, simple, and highly sensitive tool to conduct the assay. These kits contain all the components needed to probe and detect 5 whole blots.

Materials provided with the kit

- 100 µl HRP-labeled Anti-Rabbit Secondary Antibody (4^{o} C)
- 100 µl HRP-labeled Anti-Mouse Secondary Antibody (4^{o} C)
- 40 ml of 2x Blocking buffer (4^{o} C)
- 40 ml pf 5x Diluting buffer (4^{o} C)
- 40 ml 10x Washing buffer (RT)
- 5 Detection sheets
- 2.5 ml Substrate A (4^{o}C)
- 2.5 ml Substrate B (4^{o}C)

Materials and equipment needed

1. Primary Antibody (WA-0XXX or WA-1XXX, Signosis)
2. Shaker
3. Imaging system or X-ray film

Reagent preparation before experiment

- Dilute the 2x Blocking buffer, the 5x Diluting buffer, and the 10x Washing buffer to 1x buffer with ddH_2O

Assay Procedure

1. Blotting

(1) After performing sample preparation, gel electrophoresis, and transfer, block membrane in blocking buffer overnight at 4° C with shaking.
(2) Rinse with washing buffer for 5 minutes with shaking.
(3) Incubate with primary antibody (1:1000) in diluting buffer with shaking.
(4) Rinse with washing buffer for 10 minutes, 5 minutes, and 5 minutes with shaking.
(5) Incubate with secondary antibody (1:5000) in diluting buffer for 1 hour or more with shaking.
(6) Rinse 3 times with washing buffer for 10 minutes each with shaking.
(7) The membrane is ready for developing.

2. Detection

(1) Slightly dry the membrane by touching a corner of the membrane on a paper towel.
(2) Mix 0.5 ml of Substrate A and 0.5 ml of Substrate B.
(3) Place the membrane on the bottom side of detection sheet on a flat surface, and overlay the membrane with 1ml of substrate solution. Ensure that the substrate is evenly distributed over the membrane.
(4) Gently place the top side of detection sheet over the membrane without trapping air bubbles in the membrane.
(5) Incubate at room temperature for 5 minutes.
(6) Remove excess substrate by gently applying pressure over the top sheet using a paper towel.
(7) Expose the membranes using either Hyperfilm (2-10 min) or a chemiluminescence imaging system (i.e., FluorChem imager from Alpha Innotech). With either method, experiment with different exposure times.

Sample of TF Western Blot analysis