Western Blot – ECL Development Protocol

For Research Use Only

INTRODUCTION

This protocol uses a sensitive nonradioactive chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots exposed to X-ray film.

REAGENTS NEEDED

Reagent	
10x PBS	Dissolve the following in 800 mL deionized H_2O: 80 g NaCl 2.0 g KCl 14.4 g Na_2HPO_4 2.4 g KH_2PO_4 Adjust pH to 7.4 with HCl. Bring final volume to 1 L with deionized H_2O.
Blocking Buffer	1x PBS 5% Milk
Antibody Dilution Buffer	1x PBS 5% Milk 0.015% Tween-20
Wash Buffer	1x PBS 0.015% Tween-20
ECL Substrate	We recommend using Pierce® ECL Western Blotting Substrate (Cat. No. 32106, 32109, 32209)

MATERIALS NEEDED BUT NOT PROVIDED

1. X-ray film
2. X-ray film developer
3. X-ray film fixer
4. Red safelight
5. Trays for developer, fixer and deionized H_2O
6. Plastic wrap or plastic page protector

PROCEDURAL NOTES

1. Blocking the membrane is not required if working with dried PVDF membrane.
2. Always wear gloves or use clean forceps when handling membranes.
3. Do not remove film from packaging with the lights on.

PROTOCOL

1. Remove the blot from transfer apparatus and place in Blocking Buffer. Incubate at room temperature (RT) with shaking for 1 hour or overnight at 2-8°C without shaking.
2. Dilute the Primary Antibody in Antibody Dilution Buffer according to researcher's needs. A good starting dilution is 1:1000. The volume should be sufficient to cover the membrane.
3. Remove the membrane from the Blocking Buffer and place in the diluted Primary Antibody solution. Incubate at RT for 1 hour with shaking or overnight at 2-8°C without shaking.
4. Place the membrane in Wash Buffer and shake for 5 minutes. Repeat 3 times.
5. Dilute the HRP-conjugate with Antibody Dilution Buffer according to researcher's needs. A good starting dilution is 1:20,000. The volume should be sufficient to cover the membrane.
6. Wash the membrane as in step 4.
7. Prepare a Working Solution of the Pierce® ECL Western Blotting Substrate by mixing equal parts of Detection Agents 1 and 2. It is recommended to use 0.125 mL of Working Solution per cm^2 of membrane.
8. Incubate the membrane in the Working Solution for 1 minute at RT.
9. Remove the membrane from the Working Solution and place it in a plastic page protector or plastic wrap. Remove any excess liquid or bubbles with an absorbent tissue.
10. Turn off the lights (except for safelights), and expose the protected membrane to the X-ray film. The exposure time may vary. A good starting point is a 1 minute exposure.
11. Develop the X-ray film using appropriate developing and fixing solutions.

The above procedure is intended for use as a reference. Optimal concentrations, experimental conditions and experimental processes are to be determined by the individual user. No guarantee of performance using the above procedure is expressed or implied.