WEST-Queen™

Western Blot Detection System

RUO *Research Use Only* | REF *16026* | Σ *200ml* | *2℃* | *8℃*

DESCRIPTION

- The WEST-Queen™ Western Blot Detection System is enhanced chemiluminescence reagent for detection of horseradish peroxidase (HRP) conjugates on Western blots.
- The WEST-Queen™ Western Blot Detection System is designed for a high quality and long lasting chemiluminescent reaction.
- Caution : Avoid mistakenly mixing Substrate Solution and Enhancer Solution while pipetting.

INTRODUCTION

WEST-Queen™ Western Blot Detection System is a light emitting non-radioactive method for detection of immobilized specific antigen in chemiluminescent Western blots through horseradish peroxidase (HRP) labeled antibodies. Overlaying an X-ray film or visualization with a CCD camera is employed to detect luminescence corresponding to the protein band labeled by the antibodies.

KIT CONTENTS

Label	Amount
WEST-Queen™ Substrate Solution	100 ml
WEST-Queen™ Enhancer Solution	100 ml
Manual	1 ea

STORAGE AND STABILITY

- **Storage** : On receipt, all components should be stable at 2°C to 8°C, All reagents are sensitive to prolonged exposure to light. Always store the individual reagents in the light-tight containers in which they are provided. Do not freeze.
- **Stability** : The components are stable for at least 1 year when stored under the recommended conditions.

CAUTIONS

- Avoid ingestion, eye and skin contact. The Substrate Solution contains irritants and components that can be toxic when exposed to the skin. Use gloves and eye protection.
- All equipments (especially metallic device – scissors, tweezers) must be clean and free of contaminants.

ADDITIONAL REQUIRED EQUIPMENT

- Plastic wrap (enough to cover the blot)
- Scissors
- Tweezers
- Tape
- Several pieces of X-ray film
- Film cassette
- Paper backing (size of film cassette)
- Screw cap tube (or container)

NOTICE BEFORE USE

WEST-Queen™ Western Blot Detection System is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. WEST-Queen™ Western Blot Detection System is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals.

IMPORTANT NOTES BEFORE STARTING

- For best results, it is important to optimize all system components including sample amount, antibody concentrations and the membrane and blocking reagents.
- The required antibody concentrations are more dilute than those used with colorimetric HRP detection. In order to optimize the appropriate concentrations, perform a systematic dot blot analysis.
- Using the optimized blocking buffer can increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent.

PROTOCOL

To achieve the optimal signal to background ratio, it is essential to optimize concentrations of both primary and secondary antibodies.

1. Wash membrane (PVDF or nitrocellulose) three times for 5 minutes each with TBS-T Note : Do not allow the membrane to dry.
2. Mix equal quantities of Substrate Solution and Enhancer Solution by inversion in a screw cap tube (or container).

   Caution : Avoid mistakenly mixing Substrate Sol. And Enhancer Sol. While pipetting.
3. Add the mixed solution to the membrane, protein side facing up. And gently shake the screw cap tube (or container) briefly to ensure the membrane is evenly covered by mixed solution.
4. Incubate for 1-5 minute at RT without agitation.

   Note : Incubation for one minute is sufficient for detection of specific proteins. Less specific proteins may require slightly longer incubation time.
5. Drain off the excess detection reagent by holding the membrane vertically and touching the edge of the membrane with tissue to remove the excess solution. Use caution not to wipe or smear the membrane surface.
6. Wrap the membrane in plastic wrap and gently smooth out air pockets.
7. Place the membrane in a film cassette, protein side up.
8. Switch off the light and carefully place a sheet of auto-radiographic film on the top of the membrane, close the cassette and expose for an appropriate time.

   Note : Do this step in a dark room using red safe light. For abundant proteins, 30 seconds to 5 minutes should be sufficient for adequate exposure. More dilute for mulations will require longer exposure time. The appropriate exposure time should be determined by the end user.

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may frequently arise. The scientists at iNtRON are always happy to answer any questions you may have about the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
Weak or no signal	
1) Extracted protein's storage condition 2) Transfer	• Check the extracted protein's storage condition. After electrophoresis, check protein transfer by staining the gel. • Check that transfer equipment is working properly and that the correct procedure has been followed. Check protein transfer by staining the gel or membrane.
3) Insufficient concentration of antigen or antibody 4) Exposure time	• Increase concentration of antibody or antigen. • Film exposure time may have been too short
High background	
1) Too much HRP in the system 2) Inadequate washing 3) Concentration of Tween 4) Membrane's condition	• Dilute HRP-conjugate at least 10-fold. • Increase length, number or volume of washes. • Concentration of Tween used in the blocking agent was not sufficient for the application performed. • The membrane was allowed to dry during some of the incubation
White band on the film	
1) Antibody concentration Protein concentration	• White band generally occur when protein target is in excess and antibody concentration is too high.

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