Miracle-Star™
Western Blot Detection System

The Instruction Manual for Sensitive and Strong Chemiluminescent Western Blot Assay

RUO *Research Use Only* REF *16028* Σ 200ml 2℃ 8℃

DESCRIPTION

The **Miracle-Star™ Western Blot Detection System** is designed to make western target detection easier by providing the high level of sensitivity with low background in a chemiluminescent reagent system which has become one of the most common protein analysis techniques used in biochemical research. The **Miracle-Star™ Western Blot Detection System** utilizes an advanced version of the chemiluminescent HRP substrate luminol that results in fast and more sensitive detection of a target while providing a long lasting signal and high signal to noise ratio compared to any competitor's.

- The **Miracle-Star™ Western Blot Detection System** provides enhanced chemiluminescence reagent for the detection of horseradish peroxidase conjugates on western blots.
- The **Miracle-Star™ Western Blot Detection System** is designed for a high quality and long lasting chemiluminescent reaction.
- Caution : Do not use the same pipette tip for each Substrate Solution and Enhancer Solution.

CHARACTERISTICS

- **High sensitivity** : Miracle-Star™ Kit is able to detect picograms level of antigen on membrane blot.
- **High resolution** : Miracle-Star™ Kit generates high signal to noise signals
- **Steady** : Signal duration time of Miracle-Star™ Kit is longer than that of any other products.
- **Simple** : Miracle-Star™ Kit has very simple step consisted of reaction detection solution and membrane blot, and then exposure to film.
- **Fast** : Specific protein detection may be achieved within 10 minutes.

KIT CONTENTS

Label	Amount
Miracle-Star™ Substrate Solution	100 ml
Miracle-Star™ Enhancer Solution	100 ml
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STORAGE AND STABILITY

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

APPLICATIONS

- Western Blotting using Chemiluminescence (HRP Detection)

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 cleansed for free of contaminants.

ADDITIONAL REQUIRED MATERIALS

- **Completed Western blot membrane** : Nitrocellulose membrane or PVDF membranes (0.45μm pore size).
- **Dilution Buffer** : Tris Buffered Saline (TBS) or Phosphate Buffered Saline (PBS).
  TBS, pH 7.6 : 8 g NaCl, 20 ml 1 M Tris-HCl, pH 7.6, Dilute to 1000 ml with distilled water then check pH
  PBS, pH 7.5 : 11.5 g Na_2HPO_4 Anhydrous (80 mM), 2.96 g $NaH_2PO_4.2H_2O$ (20 mM), 5.84 g NaCl (100 mM). Dilute to 1000 ml with distilled water then check pH.
- **Wash Buffer** : TBS-T or PBS-T (TBS or PBS + 0.05% Tween 20)
- **Blocking Reagent** : 5% non-fat dried milk in TBS-T or PBS-T (Gelatin, Casein and Bovine Serum Albumin (BSA) may also be used as alternative blocking reagents)
- **Primary antibody** : Extremely variable, from 1:10 - 1:100,000. The optimal dilution to use depends on the specific primary antibody and the amount of antigen on the membrane.
- **HRP-conjugated secondary antibody** : commonly working dilution at 10-50 ng/ml (i.e., 1:20,000 - 1:100,000 from a 1 mg/ml stock). The optimal dilution to use varies depending on the specific conjugate and the amount of antigen on the membrane.
- **Film, Film cassette, developing and fixing reagents** : For processing autoradiographic film (X-ray film).
- **Rotary platform shaker** : For agitation of membrane during incubations.

PROTOCOL

To achieve the optimal signal to noise 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 wash buffer(TBS-T) Note : Do not allow the membrane to dry.
2. Mix thoroughly equal amounts of Substrate Solution and Enhancer Solution by inversion in a screw cap tube (or container).

   Caution : Be careful not to use the same pipette tip for each Substrate Solution and Enhancer Solution. Do not use the same pipette tip for each Substrate Solution and Enhancer Solution.
3. Add the mixed solution to the membrane transferred proteins are 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 minutes 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 vinyl wrap and gently get rid of air bubbles between membrane and vinyl wrap.
7. Place the membrane with proteins facing up in a film cassette.
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 formulation will require longer exposure time. The appropriate exposure time should be determined by the end user.

TROUBLE SHOOTING

This troubleshooting guide may be helpful in solving problems that may frequently arise. The scientists at iNtRON are always happy to answer any questions 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, according to the correct procedures. Check protein transfer by staining the gel or membrane.
3)Insufficient concentration of antigen or antibody	• Increase concentration of antibody or antigen.
4)Exposure time	• Film exposure time may have been too short.
High background	
1)Too much HRP in the system	• Dilute HRP-conjugate at least 10-fold.
2)Inadequate washing	• Increase length, number or volume of washes.
3)Concentration of Tween	• Concentration of Tween used in the blocking agent was not sufficient for the application performed.
4)Membrane's condition	• The membrane is allowed to dry during some of the incubation
White band on the film	
1)Antibody concentration Protein concentration	• White band generally occurs when protein target is in excess and antibody concentration is too high.

Technical support : +82-505-550-5600

Review date : 2017. 03.(Rev 01)