REF 16035

Σ 100ml

2 °C ~ 8 °C

Miracle-Femto™ Western Detection System

KEY FEATURE

- **Femto-grade sensitivity :** Detect target proteins with remarkable sensitivity, down to the femto grade range.
- **Broad dynamic range :** Achieve a wide linear dynamic range, enabling accurate quantification of target protein levels.
- **Rapid and simple protocol :** The easy-to-use protocol minimizes hands-on time and streamlines your Western blotting workflow.

GENERAL USE

- Solution A : Solution B = 1: 1

For research purpose only.
Not for use in diagnostic procedures for clinical purposes.

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DESCRIPTION

The Miracle-Femto™ Western Detection System, an enhanced luminol-based chemiluminescent substrate, is highly sensitive and compatible with conducting immunoblots using horseradish peroxidase (HRP)-conjugated secondary antibodies. The Miracle-Femto™ Western Detection System is designed to detect target proteins in amounts too small to be observed with typical ECL substrates. The system's excellent sensitivity and extended signal duration enable the detection of antigens down to the low femto grade range. Furthermore, the prolonged chemiluminescent signal duration allows for both digital and film-based imaging without any loss of signal. Appropriate dilutions of primary and secondary antibodies are recommended to achieve optimal signal intensity and duration. Additionally, the Miracle-Femto™ Western Detection System maintains its high performance with a 2-year shelf life when stored at refrigerated temperatures from the date of manufacture.

STORAGE

- Stable for up to 24 months at 2 ~ 8°C, do not freeze it.
- Ship at 2 ~ 8°C, beware of shipping in any condition beneath 0°C.

KIT CONTENTS

Component	Amount
Solution A (Luminol solution)	50 ml
Solution B (Hydrogen peroxide solution)	50 ml

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

REQUIRED MATERIALS BUT NOT PROVIDED

- A compatible Chemiluminescence or X-ray Imaging Systems
- A plastic sheet protector or plastic wrap to prevent the membrane from drying
- Rotary or rocking platform shaker

NOTICE BEFORE USE

- To achieve the best results, consider the following variables: sample amount, gel type, transfer method, membrane type, blocking reagent, wash buffer, primary and secondary antibody concentrations, and incubation times.
- Ensure that the membranes are fully submerged in all solutions to prevent drying out.
- Utilize a shaking or rocking platform during incubation steps for optimal performance.
- Avoid the use of sodium azide in buffers, as it inhibits HRP activity.
- Always wear gloves or use clean, plastic forceps. Any metallic devices (e.g., scissors) must be free of visible rust, as this can lead to speckling and/or high background.
- The Working Solution of the substrate is stable for 1 hour at room temperature. Limit exposure to direct sunlight or other intense light sources. Brief exposure to laboratory lighting is acceptable.

PROTOCOLS

1. **Step 1 – Wash membrane** : After protein transfer, remove the blot from the transfer apparatus and wash the membrane in deionized water for 5 minutes using agitation to remove all transfer buffer.
2. **Step 2 – Block membrane** : Block nonspecific proteins with Blocking Solution for 30 ~ 60 minutes at room temperature with shaking. Alternatively, block overnight at 2 ~ 8ºC without shaking.
3. **Step 3 – Primary antibody binding** : Incubate the membrane with primary antibody solution (0.2 ~ 10 µg/mL or follow manufacturer's recommended dilution) containing 10% blocking solution with continuous rocking for 1 hour. If desired, incubate the blot overnight at 2 ~ 8°C.
4. **Step 4 – Wash membrane** :
   1) Wash the membrane for 10 minutes using agitation with Tris-buffered saline (TBS), phosphate-buffered saline (PBS), or other physiological wash buffer containing 0.05% Tween 20 detergent.
   2) Repeat wash step 2 more times.
   3) Proceed to next step, or if using an enzyme-conjugated HRP primary antibody, proceed to Step 6.

5. **Step 5 – Add secondary antibody** : Incubate blot with the secondary antibody HRP-conjugate working dilution (0.07–1.0 µg/mL) for 30 minutes to 1 hour at room temperature using shaking.
6. **Step 6 – Wash membrane** : Wash the membrane 3 ~ 6 times for 5 minutes each in wash buffer to remove any unbound secondary antibody conjugate.
7. **Step 7 – Prepare the Working Solution :**
   Mix Solution A and B in a 1:1 ratio, and thoroughly agitate the working solution well for preparing the 0.1 ml of solution / cm² of membrane.
   - For a mini-sized membrane, 4 ml of solution is sufficient.
   - For a midi-sized membrane, 10 ml of solution is sufficient.
8. **Step 8 – Reaction with membrane : Incubate the membrane with the working solution for 1 minute.**
9. **Step 9 – Data acquisition** :
   1) Remove blot from working solution and place it in a plastic sheet protector or clear plastic wrap.
   2) Use an absorbent tissue to remove excess liquid and carefully press out any bubbles from between the blot and the membrane protector.
   3) Image the blot using an imaging system or X-ray film.

TROUBLE SHOOTING GUIDE

Problem	Cause	Solution
High Background	Over-concentrated primary or secondary antibody	• Decrease the antibody concentration.
		• Perform a dot blot to optimize the concentration.
	Insufficient wash	• Increase the frequency or duration.
	Incomplete blocking	• Decrease the antibody concentration.
		• Perform a dot blot to optimize the concentration.
No Reaction or Weak Signal	Insufficient antigen binding	• Decrease antibody concentration. • Optimize blocking reagents for achieving a balance between sensitivity and specificity.
	Poor antibody binding to the antigen	• Optimize detergent used for antibodies. • Increase the antibody incubation time

Problem	Cause	Solution
No Reaction or Weak Signal	Proteins washed from the membrane during assay	• Reduce the number or intensity of wash
	Insufficient reagent volume	• Apply additional volumes of antibody blocking reagent, or wash solution.

ORDERING INFORMATION

Cat. No	Product name	Amount
16032	Miracle-Femto™ Western Detection System	100 mL
16028	Miracle-Star™ Western Blot Detection System	200 mL
24052	GangNam-STAIN™ Prestained Protein Ladder	250 µl
21112	NomeIRT™ Western Blot Stripping Buffer	500 mL
17082	PRO-PREP™ Protein Extraction Solution (C/T)	100 mL
21071	SMART™ BCA Protein Assay Kit	2,500 T