

Product No. 07880

Chemi-Lumi One L

Features

Chemi-Lumi One is a series of high sensitive luminol-based chemiluminescence assay kits for western blotting. Chemi-lumi One L provides picogram detection of proteins with long signal duration in western blot applications.

	Chemi-Lumi One L (Product No.07880)	Chemi-Lumi One Super (Product No.02230)	Chemi-Lumi One Ultra (Product No.11644)
Sensitivity	picogram	mid-femtogram	low-femtogram

Components

Reagents	Main composition	Package
Solution A	Luminol solution	brown plastic bottle
Solution B	Peroxide solution	white plastic bottle

Required reagents

- Washing buffer : Use either a) or b).

a) 0.05% Tween 20 in TBS (TBS-T)

Mix 50ml of Tris Buffered Saline (10x)(pH 7.4)(Product No.35438), 0.25g of Polyoxyethylene Sorbitan Monolaurate (Tween 20) and distilled water to a final volume of 500ml.

b) 0.05% Tween 20 in PBS (PBS-T)

Mix 50ml of Phosphate Buffered Saline (10x)(pH 7.4) (Product No.27575), 0.25g of Polyoxyethylene Sorbitan Monolaurate (Tween 20) and distilled water to a final volume of 500ml.

- Blocking buffer : Use one of the followings.

a) Blocking One (Product No.03953)

b) Blocking One P (Product No.05999) for phosphoprotein detection.

c) 2-5% skim milk in TBS-T or PBS-T.

d) 2-5% BSA in TBS-T or PBS-T.

- Dilution buffer : Use one of the followings.

a) TBS-T or PBS-T

b) 5% Blocking One in TBS-T or PBS-T : recommended for reducing high background.

c) Signal Enhancer HIKARI (Product No.02770) : recommended for increasing weak signal.

Protocol

(Following protocol is an example. Modify accordingly if necessary.)

1. Transfer proteins to membrane from electrophoresis gel, and wash the membrane with Washing buffer for 5 minutes. Repeat twice.
2. Block the membrane with Blocking buffer.
3. Wash the membrane with Washing buffer for 5 minutes. Repeat twice.
4. Incubate the membrane with a primary antibody at room temperature for 1 hour.
5. Wash the membrane with Washing buffer for 5 minutes. Repeat twice.
6. Incubate the membrane with a HRP-conjugated secondary antibody at room temperature for 1 hour.
7. Wash the membrane with Washing buffer for 5 minutes. Repeat twice.
8. Prepare the Working solution. Mix Solution A and Solution B in one to one ratio. (Required volume is 0.125ml/ cm² membrane.)
9. Remove Washing buffer from the membrane carefully with paper towels.
10. Spread a plastic wrap on the desk, and place the membrane protein side up on it. Cover the membrane completely with the Working solution and incubate for 1 minute.
11. Remove the Working solution from the membrane carefully with paper towels, and envelop the membrane with a new plastic wrap.

Note: Carefully remove all air bubbles and wrinkles from the enveloped membrane.

12. Detection

X-ray film

In a dark room, place the enveloped membrane protein side up on a film cassette. Place an X-ray film on it and expose it for 3 minutes (optimize the exposure time if necessary).

CCD imager

Set the enveloped membrane protein side up in CCD imager, and operate it according to the user manual.

Attention

- Equilibrate the reagents to room temperature before preparing Working solutions.
- Wear gloves and use tweezers when handling blotting membrane.
- The dilution factor should be optimized for each antibody. The following dilution ranges are recommended.
Primary antibody : (1:1,000 - 1:5,000)
Secondary antibody : (1:20,000 - 1:100,000)
- If the signal is weak or too strong, optimize the exposure time first. If no improvement is observed, optimize the concentration of antibodies and sample proteins.

Cautions

Wear gloves and protective goggles because the product includes corrosive material.

Storage

Refrigerator, Protect from light

Expiration

One year from manufacturing. Expiration date is stated on the product label (Exp. yy / mm).

Packing

1KIT (Product No.07880-70) (Solution A 250ml x 1, Solution B 250ml x 1)(for 4,000 cm²)

2x50ml (Product No.07880-54) (Solution A 50ml x 1, Solution B 50ml x 1)(for 800 cm²)