# ECLong

Cat No.: SM801-0500 Size: 500 ml
Cat No.: SM801-0020 Size: 20 ml
Store at 4°C

## Description

The principle of ECLong is based on chemiluminescence and is very convenient to detect the Horseradish peroxidase (HRP) activity in many assays such as Western blotting, Southern blotting, and Northern blotting. HRP catalyzes the chemiluminescent oxidation of cyclic diacylhydrazides such as luminol by hydrogen peroxide ($H_2O_2$). ECLong can enhance the luminol-dependent chemiluminescence and be wildly used to detect the present of HRP-conjugated antibodies or streptavidin which binding to antigen or nucleotide sequence respectively.

## Features

- Signal Duration: 12 hours
- Detection Method: X-ray film or imaging acquisition system
- Suggested Antibody Dilution:
  Primary: 1/1,000 – 1/50,000
  Secondary: 1/50,000 – 1/250,000
- Lower Detection Limit:
  Low-Picogram ($10^{-14}$)
  High-Zeptomole ($10^{-19}$)

## Application

- Western blotting

## Kit Contents

| Contents | SM801-0500 | SM801-0020 |
|---|---|---|
| Reagent A | 250 ml | 10 ml |
| Reagent B | 250 ml | 10 ml |

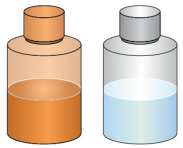
## Quality Control

The quality of the ECLong is tested on a lot-to-lot basis to ensure consistent product quality.

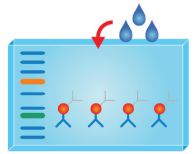
## Required Materials

- Saran wrap
- Safety light
- Cassette

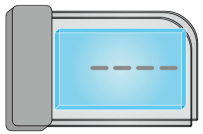
## Protocol



1. Mix the reagent A 1：1 with reagent B in ECLong and incubate the mixture for 1 min in room temperature.



2. Add the sufficient mixture solution to cover the membrane (0.1 ml/cm$^2$).
3. Incubate the membrane for 1 min in room temperature.
4. Discard the excess mixture in membrane and wrap the membrane in saran wrap. Carefully and gently remove the air bubbles from the membrane.



5. Place the membrane in the film cassette and keep the protein side up. Turn off the lights and use safety light. Then place a sheet of film on the membrane and close the cassette and expose for 10-90 seconds.
6. Open cassette and transfer the exposed film to developing machine. Then place a new film on the membrane and expose again.
   **Note:**
   The exposure time of second film can be adjusted by the intensity of first film. If the intensity was too high, please wait up to 10 minutes before re-exposing.

## Troubleshooting

Please refer to the table below to solve problems you may encounter with western blotting protocols.

| Problem | Possible Cause | Solution |
|---|---|---|
| High Background | High concentration for antibody | To optimize/Apply the lower concentration of antibody. |
| | The gathered secondary antibody | To apply 0.2 um nylon membrane / change fresh secondary antibody. |
| | The temperature is too high in the antibody incubation process | Incubated at 4°C. |
| | Secondary antibody has happened nonspecifically bind or cross reaction with Blocking Solution | Setup the control group for secondary antibody (not added primary antibody) or to reduce the concentration of secondary antibody. |
| | Primary antibody or Secondary antibody cross reaction with Blocking Solution | To add the Tween-20 into the wash buffer during the incubation, to avoid the cross reaction. |
| | Unsuitable Blocking Solution | To choose and apply the difference Blocking Solution. |

| Problem | Possible Cause | Solution |
|---|---|---|
| High Background | Not completed on the blocked | To optimize Blocking Solution.<br>To increase the concentration of protein in blocking solution.<br>To optimize the time and temperature when incubation. (Incubate 2 hours. keep at RT, if you would like to incubate for overnight, please keep at 4°C).<br>To add Tween -20 to Blocking Solution and final concentration at 0.05%. |
| Lower signal/ No signal | Antibody cross reaction with the other proteins | To choose and apply the difference blocking solution and do not use non-fat milk to block on the membrane in the system of Biotin/avidin. To reduce the concentration of secondary antibody. To test and inspect the cross reaction between the membrane and secondary antibody. |
| | Not completed on the process for transferring of membrane | Make sure it was completed activity between gel and membrane when the process of transferring. Apply gel and membrane on one filter paper, and do not reuse. It should be has a correct and complete assembling on electrophoresis process. To process the membrane following the protocol. To avoid the high temperature in the electrophoresis. Apply the positive control group or pre-stained marker. Ideal transferring time and electric current. Make sure the sample do not damage when process. |
| | Not completed on assembling of protein and membrane | Add 20% methanol to buffer of transfer membrane. Apply a small-bore / low molecular weight membrane. |
| | Antigen cover by Blocking Solution | Try to apply difference Blocking Solution. Ideal the protein concentration in Blocking Solution.<br>To shorten the blocking time. |
| | The Blocking Solution with NaN3 | Remove NaN3. |
| | The short exposed time | To extend the exposed time. |
| | The biodegradation has happen during the process of stored protein | Re-prepare new sample. |
| | The gelation for protein on membrane | Some of blocking solution may be result in the active degradation on protein. |

| Problem | Possible Cause | Solution |
|---|---|---|
| Lower signal/ No signal | The concentration was too low for Primary antibody or and Secondary antibody | Increase the concentration of antibody, and extend the incubate time. |
| | Primary antibody or Secondary antibody cross reaction with Blocking Solution | Using the Tween-20 when blocking or change the Blocking Solution (non-fat milk, BSA, serum and gel in common usage). |
| | The sample without target protein or the lower target protein on sample (unefficient antibody) | Setup the positive control group. If it run an absolute result for control group, and the sample maybe has not including target protein or the contents of target protein too low. For the lower target protein, please increase the sample to 20-30 ug per well at least, and apply protease inhibitor when prepare sample, or extract target protein by classification. |
| | Not completed on the process for transferring of membrane, or overuse on the wash of membrane | To test the efficiency of transfer membrane by Ponceau S, the PVDF membrane need to soak completed and following the correct process when transferring, do not overuse on the wash of membrane. |
| | Molecular weight for target protein are less than 10,000 | Apply a small-bore / low molecular weight membrane. To shorten the transferring time. |
| | The concentration of methanol are too high | The high concentration of methanol will result the division of protein/SDS complex and protein precipitation, in the meanwhile the gel will become solid and traction. The high molecular weight protein will be inhibited in transferring. Please decrease the concentration of methanol or apply alcohol or isopropanol to instead. |
| Nonspecific band | Nonspecific combination of SDS and protein on membrane | Wash, after transferring completed. Do not use SDS. |
| | The protein of sample has degraded | Using fresh preparing sample and apply protease inhibitor. |
| | Antibody do not for purification | Using single clone or antibody with purification. |
| | The concentration was too high for Primary antibody | Decrease the concentration of primary antibody without reducing sensitivity. |

## Caution

1. During operation, always wear a lab coat, disposable gloves, and protective equipment.
2. All products are for research use only.