

ECLong

Cat No.: SM801-0500
Cat No.: SM801-0020
Store at 4°C

Size: 500 ml
Size: 20 ml



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 (H₂O₂). ECLong can enhance the luminol-dependent chemiluminescence and be widely used to detect the present of HRP-conjugated antibodies or streptavidin which binding to antigen or nucleotide sequence respectively.

Feature

- 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-Septomole (10⁻¹⁹)

Application

- Western blotting

Kit Contents

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

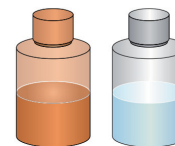
Quality Control

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

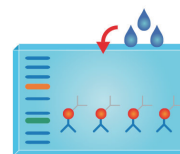
Required Materials

- Saran wrap
- Safety light
- Cassette

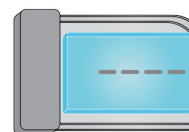
Protocol



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/cm²).
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. To increase the concentration of protein in blocking solution. 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). 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. 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.
Nonspecific band	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 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. Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.