Biomate Enhanced Chemiluminescence Detection Kit

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Cat. No. & Components	BE-10-50 Reagent A 25 mL Reagent B 25 mL	BE-10-200 Reagent A 100 mL Reagent B 100 mL	BE-10-500 Reagent A 250 mL Reagent B 250 mL

Product Description

Biomate Enhanced Chemiluminescence Detection Kit is a very convenient tool to detect the horseradish peroxidase (HRP) activity in many assays including Western Blot, Southern Blot and Northern Blot. HRP catalyzes the chemiluminescent oxidation of cyclic diacylhydrazides, such as luminol, by hydrogen peroxide (H_2O_2). Biomate Enhanced Chemiluminescence Detection Kit can enhance the luminol-dependent chemiluminescence produced, and thus be used to detect various HRP conjugates, including antibodies and streptavidin which bind to antigen or nucleotide sequence respectively.

- **Signal Duration: 6 - 8 hours**
- **Detection Method: X-ray film or imaging acquisition systems**
- **Suggested Antibody Dilution: Primary: 1:1,000 - 5,000**
  Secondary: 1:20,000 - 100,000
- **Sensitivity: between low-picogram and high-femtogram**

Storage

2 - 8 °C

Protocol

① Mix Reagent A and Reagent B by 1:1 and incubate the mixture for 1 minute at room temperature.

② Add the sufficient mixture to cover the membrane (about 0.1ml/cm^2). Incubate the membrane in the mixture for 1 minute at room temperature.

③ Remove the membrane from the mixture and wrap it by plastic wrap (ex. Saran™). Carefully and gently press out any air bubble between the membrane and the surface of the plastic wrap.

④ 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, close the cassette, and expose for 10-90 seconds.

⑤ Open cassette and transfer the exposed film to the developing machine. Then place a new film on the membrane and expose again.
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 & Tips

High Background

Possible Cause	Solution
Antibody concentration is too high	◆ Optimize/Apply the antibody with lower concentration
Aggregation of secondary antibody	◆ Filter by 0.2um nylon membrane before use / change fresh secondary antibody.
Incubation temperature is too high with antibodies	◆ Incubate at 4 °C
Secondary antibody produces nonspecific binding or cross reaction with Blocking Solution	◆ Set the control group for secondary antibody (no primary antibody) to confirm ◆ Decrease the concentration of secondary antibody
Primary or secondary antibody cross-reacts with blocking solution	◆ Switch to the optimal blocking solution, ex. normal serum instead of BSA or milk
Unsuitable blocking solution	◆ Choose and apply the difference blocking solution
Incomplete blocking	◆ Optimize the blocking solution ◆ Optimize the time and temperature when incubation (Incubate 2 hrs. at RT. If you would like to incubate for overnight, please keep at 4 °C.) ◆ Add Tween-20 into the blocking solution with the final concentration of 0.05% ◆ Add Tween-20 into the diluted antibody with the final concentration of 0.05%
Not enough time for blocking	◆ Extend the blocking time.
Incomplete washing	◆ Extend the wash time and increase the frequency and/or the volume of wash solution ◆ Add Tween-20 into the wash solution with the final concentration of 0.05%
Exposure time is too long	◆ Shorten the exposure time
Membrane problem	◆ Use clean tweezers and gloves when process ◆ Use the new membrane ◆ Apply enough solution to wet the membrane and keep it wet anytime ◆ Avoid overlapping the membranes ◆ Be careful while handling and do not damage the membrane ◆ NC membrane may have lower background than PVDF
Contamination of buffers/solutions	◆ Use fresh buffers/solutions
Pollution of instruments	◆ Make sure to keep all instruments clean ◆ Make sure no remaining gel on the membrane

Weak signal/No signal

Possible Cause	Solution
Incomplete membrane transfer	◆ Apply gel and membrane on one filter paper, and do not reuse. Ensure the system assembled correctly for electrophoresis ◆ Process the membrane by the protocol ◆ Avoid high temperature when performing electrophoresis ◆ Apply the positive control group or prestained markers ◆ Optimize the time and electric current for transfer ◆ Make sure samples undamaged during the process ◆ Ponceau S stain can be used to confirm the transfer efficiency
Proteins transfer unsuccessfully onto the membrane	◆ Add 20% methanol to buffer for transfer ◆ Use the membrane with smaller pores (ex. 0.2um instead of 0.45um) for proteins with smaller size
Antibody concentration is too low	◆ Optimize/Apply the antibody with higher concentration
Insufficient antigen	◆ Increase the sample volume, or use the concentrated sample
Antigen cover by blocking solution	◆ Try the difference blocking solution ◆ Optimize the blocking solution ◆ Shorten the blocking time
Blocking solution contains NaN_3	◆ Remove NaN_3 (the HRP inhibitor)
Exposure time is too short	◆ Extend the exposure time
Reaction time for substrate is too short	◆ Incubate with the substrate for at least 5 mininutes
Protein gelation on membrane	◆ Optimize the blocking solution, since some blocking solutions may cause such protein degradation
Biodegradation occurs during the storage process of protein	◆ Re-prepare the samples
Primary or secondary antibody cross-reacts with blocking solution	◆ Switch to the optimal blocking solution, ex. using normal serum instead of BSA or milk
Sample contains no or insufficient target protein	◆ Set the positive control group ◆ If the insufficiency is confirmed, apply protease inhibitor when preparing the sample, extract target protein by classification, and/or concentrate the sample to ensure to have at least 20-30ug protein per well/lane
Over-wash	◆ Optimize the washing time and frequency
Over-blocking	◆ Use 0.5% or even less concentrated skim milk, or try the different blocking solution ◆ Reduce the blocking time
Inefficiency of primary antibody	◆ Apply fresh antibody, and aliquot for storage ◆ Avoid freezing and thawing cycles
Secondary antibody contains NaN_3	◆ Avoid having NaN_3 (the HRP inhibitor) in all related solutions or buffers
Inefficiency of enzyme or substrate	◆ Mix the enzyme and substrate directly. The enzyme may be inactive if the coloration is not produced ◆ Use the fresh and active enzyme, as well as the fresh substrate
Membrane was not soaked completely	◆ Prewet the transfer membrane with 100% methanol and soak it thoroughly
Molecular weight of target protein < 10,000 Dalton	◆ Use the membrane with smaller pores (ex. 0.2um instead of 0.45um) for proteins with smaller size ◆ Shorten the transferring time

Nonspecific band

Possible Cause	Solution
SDS nonspecifically binds to protein on membrane	◆ Wash thoroughly after transferring ◆ Do not use SDS
Sample biodegradation	◆ Re-prepare the samples
Unpurified antibody	◆ Use single clone or purified antibody
Antibody concentration is too high	◆ Optimize/Apply the antibody with lower concentration
Too much sample	◆ Decrease the sample loading volume