NZY Advanced ECL

Chemiluminescent Substrate for Western Blotting

Catalogue number: MB40201, 2 x 125 mL

Description

NZY Advanced ECL is a highly sensitive and improved kit for chemiluminescent detection in western blotting (WB) applications. It consists in two solutions appropriate to WB with horseradish peroxidase (HRP)-conjugated antibodies. The substrate is recommended for the detection of medium to low abundant proteins. X-ray film or other imaging methods may be used to visualize the target proteins.

Components

Luminol-Enhancer Solution, 125 mL (brown bottle)
Peroxide Solution, 125 mL (white bottle)

Membrane coverage

This package allows the coverage of 2500 cm^2 of membrane area.

Storage temperature

NZY Advanced ECL should be stored at room temperature, protected from light, and is stable for one year.

Important guidelines

X-ray film exposure times should be determined for each antibody system
The usage of blocking buffer to dilute antibodies may reduce background and increase sensitivity
Sodium azide in blocking buffers or wash solutions inhibits HRP activity
To prevent high background in the blots, always wear gloves and use tip forceps when handling membranes
Rusty objects (scissors or forceps) may create undesirable artefacts or high background areas

Chemiluminescent Detection Protocol

Blot Size (cm)	HRP Substrate Required
Mini membrane 7 × 8.5	6 mL
Midi membrane 8.5 × 13.5	12 mL

1. Place the blotted membrane with the protein-side up in a container or clear plastic sheet protector, and add a working solution of the Luminol-Enhancer Solution and Peroxide Solution (1:1 ratio) of the NZY Advanced ECL onto the blot. A freshly prepared working solution is preferred.
2. Incubate for 2 to 5 minutes at room temperature.
3. Remove the excess substrate and proceed with the imaging of the membrane - x-ray film (1 to 5 minutes) or digital imaging system.

The chemiluminescent signal on the blot will last for about 1 hour. If necessary, fresh substrate can be added to the same blot for consecutive exposures.

NZY ECL Products

NZY ECL	Standard	Advanced
Signal intensity	Medium	High
Signal duration	Medium	Longer
Protein amount	High	Medium
Primary antibody	1:500 - 1:5000	1:1000 - 1:15000
Secondary antibody	1:20000 - 1:100000	1:25000 - 1:150000

Troubleshooting

Weak or no signal

Cause:

- Antibody concentration or incubation time may be too low
- Blocking buffer might be inadequate
- Washes might be too stringent
- Low amount of target protein

Solution:

- Expose to x-ray for longer period of time
- Increase antibody concentration and/or incubation time
- Try a different blocking reagent (e.g. Nonfat Milk - MB260; Albumine Bovine Fraction V (BSA) - MB046)
- Decrease the number and/or duration of the washes
- Increase amount of added sample
- Make sure protein transfer was efficient: confirm protein transfer by Ponceau S staining (MB192) of protein sample, and/or by protein ladder marker transfer (NZYColour Protein Marker I – MB215; NZYColour Protein Marker II – MB090)

Strong signal quickly disappears

Cause:

- High HRP-Antibody concentration may have exhausted the substrate prematurely
- ECL excess may cause substrate depletion

Solution:

- Decrease antibody concentration significantly
- Decrease amount of ECL added to the membrane

High background or nonspecific bands

Cause:

- Antibody concentration may be too high
- Blocking might be incomplete
- Washes might be insufficient
- Excess of protein sample loaded
- Used solutions may be contaminated

Solution:

- Expose to x-ray for shorter period of time
- Decrease antibody concentration
- Increase the incubation/concentration of blocking buffer
- Increase the number, volume and/or duration of the washes; increase the concentration of Tween-20 in the washing buffer
- Reduce amount of protein sample
- Filter the solutions prior to use
- Make sure that the membrane is hydrated at all times
- Drain excess ECL substrate

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