MB40101_IFU_EN_V2401

NZY Standard ECL

Catalogue number	Presentation
MB40101	2 x 125 mL

Description

NZY Standard ECL is a 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 high to medium abundant proteins. X-ray film or other imaging methods may be used to visualize the target proteins.

Shipping & Storage Conditions

This product is shipped at room temperature and should be stored at 15ºC to 25ºC protected from light. The product will remain stable till the expiry date if stored as specified.

Components

COMPONENT	BOTTLE	VOLUME
Luminol-Enhancer Solution	brown	125 mL
Peroxide Solution	white	125 mL

Specifications

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

Sensitivity: The usage of blocking buffer to dilute antibodies may reduce background and increase sensitivity.

Standard Protocol

Recommendations before starting

- X-ray film exposure times should be determined for each antibody system.
- Warnings: Sodium azide in blocking buffers or wash solutions inhibits HRP activity.
- Handling instructions: 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.

Procedure for chemiluminescent detection

1. Place the blotted membrane with the protein-side up in a container or clear plastic sheet protector, add a working solution of the Luminol-Enhancer Solution and Peroxide Solution (1:1 ratio) of the NZY Standard 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.

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

Notes

- *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.*

Technical notes

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

Troubleshooting is often a systematic, meticulous process where varying one parameter at a time and evaluating impacts can unveil the root cause of issues. These adjusted suggestions, incorporating a blend of specificity and exploratory approaches, aim to enhance the clarity and actionability of your troubleshooting guide. Should any other technical or procedural aspects require attention, your feedback and additional information will always be welcomed.

- **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; Bovine Serum Albumin (BSA) – MB470)
- 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 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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