NZY Supreme ECL HRP Substrate

Catalogue number: MB19301, 100 mL

Description

NZY Supreme ECL HRP Substrate is an improved reagent for chemiluminescent detection in western blotting (WB) applications. It consists in a ready-to-use premixed solution 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.

Membrane coverage

This package allows the coverage of 1000 cm^2 of membrane area.

Storage temperature

NZY Supreme ECL HRP Substrate should be stored at 2-8 °C. There is no need to protect the reagent from light.

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

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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 the NZY Supreme ECL HRP Substrate onto the blot.
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.

Troubleshooting

High background or nonspecific bands

- Antibody concentration may be too high
- Blocking might be incomplete
- Washes might be insufficient

- Expose to x-ray for shorter period of time
- Decrease antibody concentration
- Increase the incubation/concentration of blocking buffer
- Increase the number and/or duration of the washes

Strong signal quickly disappears

- High HRP-Antibody concentration may have exhausted the substrate prematurely

- Decrease antibody concentration significantly

Weak or no signal

- Antibody concentration may be too low
- Blocking buffer might be inadequate
- Washes might be too stringent

- Expose to x-ray for longer period of time
- Increase antibody concentration
- Try a different blocking reagent
- Decrease the number and/or duration of the washes

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