NuGlo (luminol) ECL Substrate Kit for Western blot

□ **Cat**. # 80215-250 **Size**: Makes 250 mls (125 mls each of Soln. A and B) **Store** at 4°C

□ **Cat**. # 80215-250-S **Size**: Makes 50 mls (25 mls each of Soln. A and B) **Store** at 4°C

Introduction

The Enhanced NuGlo™ substrate kit is a luminol based chemiluminescent detection system designed for use with peroxidase labeled antibodies immobilized on membranes. The substrate is supplied as a stable ready-to-use component solutions. Sufficient material is provided to process approximately 4000 cm^2 of membrane or 25-30 mini blots.

Background

Chemiluminescent substrates have proven to be sensitive and accurate tools for the visualization of labeled probes in membrane immunoassays. Peroxidase labeled antibodies may be detected by using colorimetric substrates such as 4-chloro-1-naphthol, diaminobenzidine or tetramethylbenzidine (4-CN, DAB, or TMB), which deposit an insoluble purple, brown or blue color directly onto the membrane. As an alternative method, radiolabeled antibodies produce a reaction visualized on X-ray film. However, the special handling required for radioisotopes increases the hazard and cost of this method. Chemiluminescent substrates provide higher sensitivity than colorimetric or ^{125}I methods without the disposal concerns associated with the use of radioisotopes. Positive reaction sites are readily detected with good resolution and minimal background. Development of the signal on the X-ray film provides a permanent record of results. ADI's Enhanced NuGlo substrate kit provides a rapid and convenient system for the detection of small quantities of peroxidase labeled antibodies, or any other peroxidase labeled tracer such as streptavidin.

Principal

Following incubation with peroxidase labeled antibody or streptavidin as the final step of a membrane immunoassay, the membrane is thoroughly washed and reacted with the chemiluminescent substrate. In the presence of hydrogen peroxide, peroxidase converts luminol to an excited intermediate dianion. This dianion emits light on return to tits ground state. The reaction is visualized using a camera luminometer, or through exposure to X-ray film.

Kit Components

The Chemiluminescent substrates have a minimum of 1 year stability when stored at 2-8°C: (discard if microbial growth is evident).

1. Chemiluminescent substrate A
2. Chemiluminescent substrate B

To prepare a working solution, mix the A & B components in equal volumes 1:1 (Example: *5 ml of soln. A + 5 ml soln B: Total 10 ml).*

Reagents required for Western Blotting but not provided in the substrate kit

Primary antibody, peroxidase labeled antibody, wash buffers, and diluents.

Note: All immunoassay reagents except primary antibody are included in the ADI **western blot Kit** for chemiluminescence. The kit contains Enhanced NuGlo substrate and your choice of peroxidase labeled antibody: Anti-human/rabbit/mouse/rat/goat/monkey IgG-HRP Conjugate.

Accessories required but nor provided

Nylon, nitrocellulose or PVDF membrane, pipettes, test tubes, reaction vessels, luminometer or X-ray film, and film processing & development supplies.

Procedure

1. Perform standard blotting and immunoassay procedure.
2. **Note**: Milk-based blocking solutions are recommended for use with Enhanced NuGlo substrate. The use of BSA or serum-based blocking reagents may cause high background in some cases.
3. Following incubation with peroxidase labeled antibody, perform at least three washes of five minutes each. (Due to the high sensitivity of Enhanced NuGlo substrate, high background can result from inadequate washing procedures.)
4. Prepare substrate by mixing equal volumes of Chemiluminescent Substrate A and Chemiluminescent Substrate B.
5. Immerse membrane in substrate solution for 2-5 minutes at room temperature.
6. Remove membrane from substrate solution and remove excess liquid. Begin signal development as soon as possible for maximum sensitivity, typically within 15 minutes.

7. Measure light emission using a camera luminometer or X-ray film. If using film, lay membrane flat between two sheets of plastic to avoid wetting the film. Be sure that the surface of the membrane to which the protein was applied is facing the film. A typical exposure time on X-ray film is from less than a minute to 15-30 min.; the optimal exposure should be determined for each application or assay system.
8. Develop film according to the manuf. instructions.

General Notes

1. Always incorporate a positive control, negative control, and reagent control (blank).
2. Use of a protein stain such as Amido black or **StainAll** to confirm transfer of protein from gel to membrane is recommended.
3. The high sensitivity of chemiluminescent substrate may allow reduction in antibody concentration or incubation times. Optimal dilution and incubation times must be determined for each application.
4. Remove as much buffer as possible after washes, but do not allow the membrane to dry.
5. Use distilled water to prepare all reagents.

Causes of Excess Signal or Background

1. Insufficient dilution of the peroxidase labeled antibody is the most common cause of high background.
2. Excessive antibody incubation times.
3. Inadequate washing or blocking procedures.
4. Excessive amounts of protein loaded on gel.
5. Excessive exposure time of the film (delay of exposure of the membrane to the film for several minutes after substrate incubation may enhance the signal-to-noise ratio).
6. Exposure of the film to light during development will cause fogging of the film.

Causes of No Signal

1. Procedure was not followed properly, a reagent may have been omitted or prepared improperly.
2. Insufficient/aberrant Protein transfer from the gel to the membrane.
3. Specificity of the peroxidase labeled antibody was not appropriate for the primary antibody.
4. Correct orientation of the membrane was not maintained throughout the procedure.
5. Presence of azide in any buffer has suppressed peroxidase activity.

Causes of Weak Signal

1. Antibody concentration was too low
2. Antibody incubation time was too short.
3. Not enough protein was loaded onto the gel.
4. The primary antibody has low affinity for the target protein. (Affinity of the antibody may change after the denaturation of the sample protein in SDS buffer)
5. Exposure time of the membrane to film was too short.
6. Reagents were not warmed to room temperature before use.

Causes of Poorly Defined Signal

1. Protein transfer was performed incorrectly; check recommendation for use of blotting apparatus.
2. Excess substrate solution was not removed from membrane prior to signal development.
3. Film or membrane shifted position during signal development , causing `ghost' images. (this can occur within a few seconds).
4. Certain types of membranes require special handling. Check for manufacturer's recommendations.

References

1. Kricka LJ. Chemiluminescent and bioluminescent techniques. Clin. Chem. 37/9:1472-1481 (1991)
2. Knect DA and Dimond RL. Visualization of antigenic proteins on Western blots. Anal. Biochem. 136:180-184 (1984)
3. Burnette WN. "Western blotting". Electrophoretic transfer of proteins and nucleic acids from slab gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated Protein A. Anal. Biochem. 102:459-471 (1981)
4. Isacsson V and Wettermark G. Chemiluminescence in analytical chemistry. Anal. Chim. Acta 68:339-362 (1974)
5. Towbin H et. al. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. Proc. Natl. Acad. Sci. USA. 76:4350-4354 (1979).
6. Reinhart MP and Malamud D. Protein transfer from isoelectric focusing gels; the native blot. Anal. Biochem. 123:229-235 (1982).

Related items

80200-Rb	Western blot Kit for Rabbit Primary
80201-Gt	Western blot Kit for Goat Primary
80202-Mo	Western blot Kit for Mouse Primary
80206-GP	Western blot Kit for G. pig Primary
80207-Hu	Western blot Kit for Human Primary
80208-Sh	Western blot Kit for Sheep Primary
80209-Pg	Western blot Kit for Pig Primary Antibodies

Secondary antibodies for rabbit, mouse, goat etc.

80215-250 110420A

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