



HyGLO™
Chemiluminescent HRP Detection Reagent

Catalog Number E2500

For Research Use Only

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Introduction

Denville Scientific's HyGLO™ Chemiluminescent HRP Antibody Detection Reagent serves as a highly sensitive substrate for the detection of horseradish peroxidase (HRP) conjugates routinely used in immunoblotting. The method provides sensitivity down to 1 picogram of antigen. The chemiluminescent signal can be detected on autoradiography film, such as HyBlot CL™ Autoradiography film.

Kit Contents

Reagent A (250mL) – Enhancer
Reagent B (250mL) – Peroxide

A volume of 500mL equates to 4,000cm² of membrane.

Storage

HyGLO™ is shipped at room temperature. Upon receipt HyGLO™ should be stored at 4°C. Do not cross-contaminate Reagents A & B as this will cause a decrease in reagent performance.

Notes

1. The HyGLO™ reagents are sensitive to prolonged exposure to light. Storage of these reagents should be in the bottles provided to ensure the activity of the kit.
2. Do not mix reagents from other kits. The HyGLO™ Reagent has been optimized within each lot.
3. Prepare the HyGLO™ reagent immediately before use. Prepare enough reagent for the area of the membrane required and discard any remainder.
4. Keep the membrane hydrated after addition of antibody solutions.
5. Do not use sodium azide as a bacteriocide as sodium azide is a potent inhibitor of peroxidase activity.
6. Accidental freezing of the substrates will not cause degradation. If frozen thaw the solutions and mix well, then store as recommended.

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Protocol

1. Prepare HyGLO™ by mixing equal volumes of Reagent A and Reagent B. Enough HyGLO™ should be prepared to completely cover the membrane.

TIP: Start with 5mL total HyGLO™ reagent per 40cm² of membrane. This is equivalent to one standard sized mini-gel immunoblot.

2. Drain excess moisture from the membrane.
3. Add the prepared HyGLO™ reagent from Step 1 to the membrane and incubate for 1 minute at room temperature.

NOTE: The 1 minute incubation is a good starting point for typical experimental conditions. This incubation time may need to be optimized for specific protocols.

4. Drain excess HyGLO™ from the membrane and wrap the membrane in clear plastic wrap.
5. Quickly place the membrane (protein side facing up) in an autoradiography film cassette.
6. In a darkroom expose the membrane to a piece of HyBlot CL™ Autoradiography Film.

TIP: Start with an initial exposure to the film of 30s – 60s. The optimal time for the exposure depends upon the amount of protein being detected and the HRP system being utilized. Exposure time can vary from a few seconds to a number of hours.

7. Develop the film and adjust the exposure time as required to obtain optimal results.

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Troubleshooting Guide

Little to no signal.

- Poor transfer of proteins.
 - Check transfer of proteins by staining the membrane and gel.
 - Check transfer equipment and repeat experiment if required.
- Membrane integrity.
 - Ensure membrane is adequately hydrated.
- Antibody concentrations are too low.
 - Titrate 1° and 2° antibody concentrations for optimal performance.
- HyGLO™ reagent improperly prepared.
 - Add HRP conjugate to HyGLO™ reagent and look for light signal to verify reaction.

Excess signal (black bands).

- Antibody concentrations are too high.
 - Titrate 1° and 2° antibody concentrations for optimal performance.
- Antigen is in excess.
 - Optimize antigen concentration.

High background.

- Antibody concentrations are too high.
 - Titrate 1° and 2° antibody concentrations for optimal performance.
- Antigen is in excess.
 - Optimize antigen concentration.
- Inadequate blocking of membrane.
 - Increase blocking agent concentration.
- Exposure to film is too long.
 - Decrease exposure time to film or let signal decay for several minutes prior to another exposure.

White (negative) bands.

- Depletion of HyGLO™ substrate due to excess antibody and/or antigen.
 - Reduce antibody concentration.
 - Optimize antigen concentration.