



Introduction

Denville Scientific's HyGLO Quick Spray™ Chemiluminescent HRP Antibody Detection Reagent combines a highly sensitive substrate for the detection of horseradish peroxidase (HRP) conjugates, routinely used in immunoblotting, with the convenience of simple spray bottle. The method provides "just in time" mixing of two reagents with the sensitivity down to 1 picogram of antigen. The chemiluminescent signal can be detected on autoradiography film, such as HyBlot CL™ Autoradiography film.

Kit Contents

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

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

Storage

HyGLO Quick Spray™ is shipped at room temperature. Upon receipt of HyGLO Quick Spray™, attach bottles to head and store assembled apparatus upright at 4°C.

Notes

1. The HyGLO Quick Spray™ 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 Quick Spray™ Reagent has been optimized within each lot.
3. Keep the membrane hydrated after addition of antibody solutions.
4. Do not use sodium azide as a bactericide as sodium azide is a potent inhibitor of peroxidase activity.
5. Accidental freezing of the substrates will not cause degradation. If frozen thaw the solutions and mix well, then store as recommended.



Protocol

1. Drain excess moisture from the membrane.
2. Spray enough HyGLO Quick Spray™ reagents to completely cover the membrane and incubate for 1 minute at room temperature.

NOTE: Adjust the spray intensity to desired amount by spinning the nozzle on the head clockwise. Even in the "stream" configuration, there is not enough force to "spray off" antibodies or proteins.

3. Drain excess HyGLO Quick Spray™ from the membrane and wrap the membrane in clear plastic wrap.
4. Quickly place the membrane (protein side facing up) in an autoradiography film cassette.

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.

5. 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.

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

Spray Nozzle Alignment: To adjust the spray intensity rotate the nozzle on the barrel of the sprayer as follows:

Fine Mist: Position the opening in the nozzle so that the opening is at the very bottom position and "SPRAY" can be read on the top of the sprayer head.

Stream: Position the opening in the nozzle so that "STREAM" can be read on the top of the sprayer head.



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 Quick Spray™ reagent improperly prepared.
 - Add HRP conjugate to HyGLO Quick Spray™ 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 Quick Spray™ substrate due to excess antibody and/or antigen.
 - Reduce antibody concentration.
 - Optimize antigen concentration.

Bubbles.

- Bubbles will not affect the activity of HyGLO Quick Spray™ as long as the membrane has been completely covered with the reagent.



HyGLO Quick Spray™ Chemiluminescent HRP Detection Reagent

Catalog Number E2400

For Research Use Only

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