SpectraQuant™-HRP CL SPRAY Western Substrate

Chemiluminescent HRP Detection Reagent

Catalog Number: WB003-500

For Research Purposes Only

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SpectraQuant™-HRP CL SPRAY Western Substrate

Introduction:

SpectraQuant™-HRP CL SPRAY Western Substrate serves as a highly sensitive chemiluminescent substrate for the detection of horseradish peroxidase (HRP) conjugates routinely used in immunoblotting. The method provides sensitivity down to 1 picogram of antigen. The reagents are compatible with nitrocellulose and polyvinylidene (PVDF) membranes. The chemiluminescent signal can be detected on autoradiography film or through use of a CCD camera.

Contents:

Reagent A (250 ml) – Enhancer
Reagent B (250 ml) – Peroxide
A volume of 500 ml equates to 4,000 cm^2 of membrane.

Setup & Storage (DO NOT SKIP)

SpectraQuant™-HRP CL SPRAY is shipped at room temperature. Upon receipt SpectraQuant™-HRP CL SPRAY, perform the following steps to prime the sprayer head:

1. Remove the sprayer head from packaging.
2. Place both siphon tubes in deionized MQ purified water.
3. Squeeze the sprayer head trigger a minimum of six (6) times. Make sure that water is being drawn up both siphon tubes before proceeding.
4. Attach both bottles to sprayer head. Make sure that the paddles at the base of the sprayer head have been rotated to the “ON” position marked on the bottles. Confirm that each of the bottles are seated it firmly against the base of the sprayer head.
5. Once the bottles are securely in place, squeeze the sprayer head a minimum of six (6) times to dram the reagents into the siphon tubes and prepare it for use.
6. Store assembled apparatus at 4°C.

Notes:

1. Do not mix SpectraQuant™-HRP CL Western Substrate reagents with reagents from other kits. The SpectraQuant™-HRP CL Western Substrate reagents have been optimized within each lot.
2. Prepare the SpectraQuant™-HRP CL Western Substrate working solution immediately before use. *(Prepare enough working solution to completely cover the area of membrane utilized.)*
3. Keep the membrane hydrated following incubations with any antibody containing solutions and prior to using the SpectraQuant™-HRP CL Western Substrate.
4. Do not use sodium azide as a bacteriocide. Sodium azide is a potent inhibitor of peroxidase activity.
5. Accidental freezing of the substrates will not cause degradation. If frozen, thaw, mix well, and store as recommended.

Protocol:

1. Drain excess moisture from the membrane after blocking.
2. Spray enough SpectraQuant™-HRP CL SPRAY reagents to completely cover the membrane and incubate for 1 minute at room temperature.
3. Drain excess SpectraQuant™-HRP CL 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.
5. In a darkroom expose the membrane to a piece of autoradiography Film.
6. Develop the film and adjust the exposure time as required to obtain optimal results.

Notes

1. Keep the membrane hydrated after addition of antibody solutions.
2. Do not use sodium azide as a bacteriocide as sodium azide is a potent inhibitor of peroxidase activity.
3. Accidental freezing of the substrates will not cause degradation. If frozen thaw the solutions and mix well, then store at 4°C. Add the prepared SpectraQuant™-HRP CL Western Substrate working solution from Step 1 to the membrane and incubate for 1 minute at room temperature.

   1 minute of incubation is a good starting point for typical experimental conditions. However, the length of incubation may need to be optimized for specific protocols.
4. Drain the SpectraQuant™-HRP CL Western Substrate working solution from the membrane and, while keeping the membrane from drying, 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 autoradiography film.

   Start with an exposure time of 30 – 60 s. Exposure times can be varied from a few seconds to a number of hours depending on the required sensitivity. Develop the film and adjust the exposure time as required to obtain the required sensitivity.

Troubleshooting Guide

Little to no signal:

- Poor transfer of proteins.
  - Check efficient transfer of proteins to the membrane by staining with SpectraQuant™-PS Green (APE-BridgePath Scientific Cat. No. PS003) or similar, membrane compatible, protein staining reagent.
- Membrane dehydration.
  - Ensure membrane is adequately hydrated throughout the detection procedure.
- Antibody concentrations are too low.
  - Titrate 1° and 2° antibody concentrations for optimal performance.
- SpectraQuant™-HRP CL Western Substrate working solution improperly prepared.
  - SpectraQuant™-HRP CL Western Substrate working solution must be prepared with equal amounts if Reagents A and B. The working solution is stable for more than 1 h at 20 ºC.

Excess signal (bands too dark or 'negative' bands):

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

High background:

- Inadequate blocking of non-specific sites on the membrane.
  - Incubate membrane for at least 30 min. in the recommended blocking solutions. *SpectraQuant™-HRP CL Western Substrate is optimized for use with Western Block™ (APE-BridgePath Scientific Cat No. WB002-1000), or 3 % Milk powder in PBS/TBS + 0.05 % Tween 20.*
- Antibody concentrations are too high.
  - Titrate 1° and 2° antibody concentrations for optimal performance.
- Antigen is in excess.
  - Optimize antigen concentration.
- Exposure to film is too long.
  - Decrease exposure time to film or let signal decay for several minutes prior to another exposure.