SpectraQuant™-HRP CL Western Substrate

Chemiluminescent HRP Detection Reagent

Catalog Number: WB001-500

For Research Purposes Only

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Introduction:

SpectraQuant™-HRP CL 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.

Storage:

SpectraQuant™-HRP CL Western Substrate is shipped at room temperature. Upon receipt, all components should be stored at 4 °C. SpectraQuant™-HRP CL Western Substrate reagents are sensitive to prolonged exposure to light. To ensure activity, store reagents in the bottles provided. Cross-contamination of Reagents A and B will reduce the performance and lifetime of the reagents.

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. Transfer antigen to a membrane using standard techniques and block non-specific sites using a suitable blocking solution such as Western Block™ (BridgePath Scientific Cat No. WB002-1000), or 3 % Milk powder in PBS/ TBS + 0.05 % Tween 20, for at least 30 min.
2. Incubate the membrane in blocking solution containing non-conjugated antibody, as required.
3. Wash membrane thoroughly with PBS/TBS + 0.05 % Tween 20 (3 x 10 min) and incubate in blocking solution containing the HRP-labeled conjugate antibody, as required.
4. Wash membrane thoroughly with PBS/TBS + 0.05 % Tween 20 (3 x 10 min) to remove any non-bound antibodies.
5. Prepare SpectraQuant™-HRP CL Western Substrate working solution by mixing equal volumes of Reagent A and Reagent B. Enough working solution should be prepared to completely cover the membrane.

   5 ml of SpectraQuant™-HRP CL Western Substrate working solution is sufficient to cover 40 cm^2 of membrane - equivalent to one standard sized mini-gel immunoblot.
6. Drain excess moisture from the membrane.
7. 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.
8. 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.
9. Quickly place the membrane (protein side facing up) in an autoradiography film cassette
10. 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 (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™ (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.

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