Photon Chemiluminescence Detect System

FIVEphoton Biochemicals Cat. Nos. PCD-250 and PCD-500

Storage: 4°C in the dark.
Safety: Avoid ingestion, skin and eye contact

Kit Contents:

1. Solution A (Luminol-ECL substrate): 250 ml (PCD-250) or 500 ml (PCD-500)
2. Solution B (Activator-Peroxide): 1ml (PCD-250) or 2 ml (PCD-500)

Overview:
The Photon Chemiluminescence Detection System is employed to identify and quantitate protein bands on Western blots using a horseradish peroxidase (HRP) detection system and chemiluminescence. The researcher first resolves proteins in SDS-PAGE gels, and then transfers the proteins to PVDF or nitrocellulose membranes. The membranes are incubated first with a "primary" and then with a "secondary antibody" that is typically conjugated with horseradish peroxidase (HRP). The primary antibody is selective to the protein (antigen) of interest on the Western blot membrane, whereas the secondary antibody is "host selective" for the primary antibody. For example, if a mouse IgG antibody is used as the primary antibody to detect the antigen on the Western blot membrane, then a secondary antibody is employed to bind to the mouse IgG antibody. The enzyme horseradish peroxidase that is conjugated to the secondary antibody reacts with the peroxidase substrate in the ECL reagent resulting in a chemical reaction that emits luminescence. Overlaying an X-ray film, or visualization with a CCD camera, is employed to detect luminescence corresponding to the protein band labeled by the antibodies.

Storage Conditions: Store all reagents at 4°C upon arrival. Shipped at ambient temperature.
Caution: Avoid ingestion, eye and skin contact. Photon Chemiluminescent Substrate contains irritants and components that can be toxic when exposed to the skin. Use gloves and eye protection.

Procedures
Perform the following procedures after the incubation of the Western blot membrane with both primary and secondary antibodies and the final washes of unbound secondary antibody.

1. Leave membrane (PVDF or nitrocellulose) in 200ml of TBS-T (Tris-buffered saline-Tween).

2. To develop a 10 cm x 10 cm or smaller Western blot membrane, prepare detection solutions A and B (5 ml solution A to 12µl solution B) at room temperature in a foil-wrapped falcon tube to make the developing solution. Limit bright light exposure. This solution can be reused for 24 hours if stored in 4°C away from light. Directly scale the volumes of solutions A and B for other size membranes.

3. Drain off TBS-T from the membrane.

4. Lay the membrane on saran wrap. Cover the entire surface of the membrane with developing solution mixed in step 2. You can use a pipettes to overlay the developing solution over the membrane.

5. After 1 min., drain off the excess detection solution and wrap the membrane in saran wrap. Avoid bubbles and liquid on the external surfaces of the saran wrap. Go to a dark room with an X-ray film developer, and expose the membrane to separate X-Ray films for different durations to empirically optimize the exposure length. Load films into a X-ray film developer machine and observe the developed Western blot film. Repeat the exposure steps using different exposure times of blot to film if needed

Troubleshooting

1. There is a dark background on the film with little resolution of the protein bands on the Western blot.
   Possible Causes: 1. The primary antibody is not selective for the antigen, or it was used at an improper titer.
   Resolution: Identify an antibody selective toward the protein of interest. Use various lengths of exposure time of the blot to film to control background darkness.

2. White blotches are observed on the film. *Possible Cause:* There were bubbles in the sandwich of the saran wrap with the blot. Resolution: Repeat the chemiluminescence development procedures, taking care to remove all bubbles.

3. Black specks were observed in the film. *Possible Cause:* Gel debris were on the blot. Resolution: Rinse the blot with PBS-T to remove gel debris and repeat the Western blot development procedures.