ECL Detection Kit

(Cat. No.: S1GNM03j20001)

Description:

ECL Detection Kit is a horseradish peroxidase (HRP) substrate and it is widely utilized for the identification of specific proteins. As shown in Fig. 1, protein samples are initially separated through SDS-PAGE and then transferred onto either nitrocellulose or polyvinylidene fluoride (PVDF) membranes. Following a blocking step, the membrane undergoes probing with a primary antibody. After a thorough washing procedure, the membrane is incubated with a secondary antibody conjugated to horseradish peroxidase (HRP) enzyme. After a final washing step, the membrane is exposed to the ECL Detection Kit to generate a detectable signal.

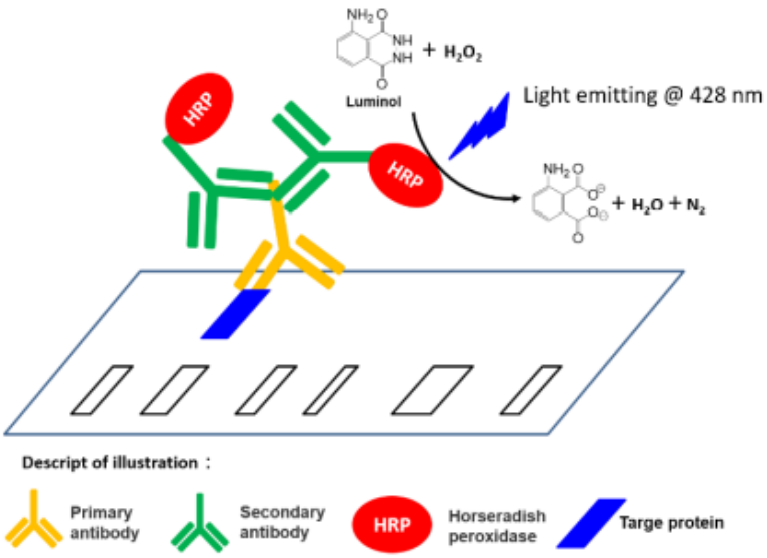

Fig 1. Principles of ECL Western blotting Protein Detection

Kit Contents:

Contents	S1GNM03j20001 (50 mL x 2)
ECL Detection Kit, Reagent A	50 ml x 1 bottle
ECL Detection Kit, Reagent B	50 ml x 1 bottle

Storage:

This product can be shipped at room temperature. For long-term storage, the ECL Detection Kit is stable for 6 months at 4°C when protected from light.

Protocol:

1. **Blocking:**
    - After protein transfer, remove the membrane from the transfer device.
    - Incubate the membrane with blocking buffer to block nonspecific binding sites for 1 hour at room temperature with shaking or overnight at 4°C without shaking.
2. **Primary Antibody Incubation:**
    - Remove blocking solution and add the diluted primary antibody solution.
    - Incubate for 1 hour at room temperature with shaking.
3. **Washing:**
    - Wash the membrane three times with TBST wash buffer (TBS with 0.05-0.1% Tween 20), each for five minutes, to remove unbound antibody.
4. **Secondary Antibody Incubation:**
    - Incubate the membrane with diluted secondary antibody solution (HRP conjugated) for 1 hour at room temperature with shaking.
5. **Washing:**
    - Wash the membrane three times with TBST, each for five minutes, to remove unbound antibody. Additional washes can help reduce background.
6. **Substrate Preparation:**
    - Mix equal volumes of Reagent A (black plastic bottle) and Reagent B (white plastic bottle) to prepare the substrate working solution.
    - Prepare only enough working solution to cover the membrane (e.g., 6-7ml per 10 X 5 cm membrane).

    Note: Use the prepared substrate working solution immediately after mixing. It remains stable for up to 1 hour at room temperature.
7. **Substrate Incubation:**
    - Incubate the membrane for 1–5 minutes at room temperature without shaking. Ensure the working solution covers the membrane.

8. **Membrane Handling:**
    - Remove the membrane from the solution, then use an absorbent towel to blot excess liquid, and place it in a plastic sheet protector.
9. **Exposure:**
    - In a dark room with a safe light, position the covered membrane in a film cassette with the protein side facing up.
    - Place the film on top of the membrane and expose it for 1 minute.
    - Exposure time can be adjusted for optimal results. Note that light emission is most intense immediately after substrate incubation, decreasing significantly within a few minutes.

◎ **Revision History** ◎

Description	Version	Date
Initial Release	S1GNM03j20001_Protocol_V1	Sep 2023