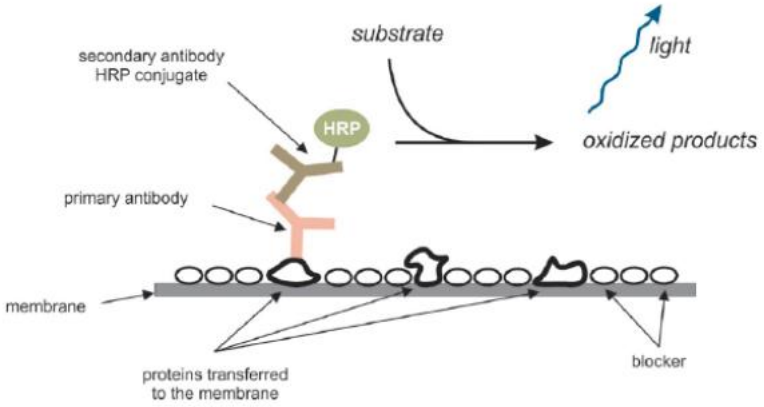
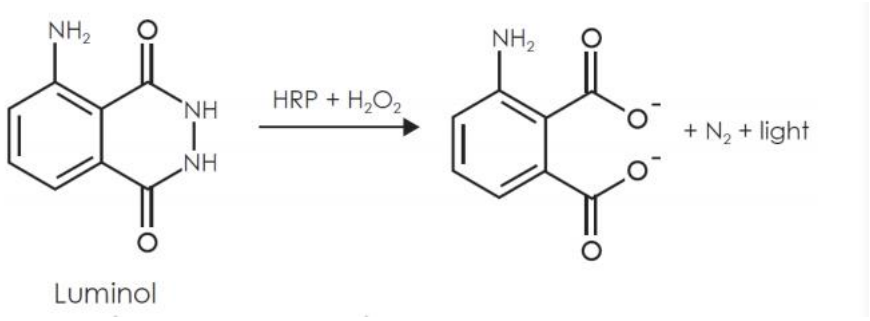
SuperLumia ECL Plus HRP Substrate Kit

Principle

SuperLumia ECL Plus HRP Substrate Kit is a highly sensitive, nonradioactive, enhanced luminol-based chemiluminescent substrate for easy detection of horseradish peroxidase (HRP) on immunoblots. The ECL Western Blotting Substrate offers superior signal and clear background. Blots can be repeatedly exposed to X-ray film to obtain optimal results or stripped of the immunodetection reagents and re-probed. The special formulation of ECALBIO SuperLumia ECL Plus HRP Substrate makes it the ideal substitute for Pierce ECL Western Blotting Substrate without the need for additional optimization of assay conditions.



The principle of chemiluminescent Western Blotting



Chemiluminescence of luminal

Assay Overview

Wash

remove all transfer buffer

⇩

proteins from gel to membrane

⇩

Block

to mask non-specific protein binding sites on membrane

⇩

Primary Antibody

binds to protein of interest

⇩

Wash

to remove excess antibody

⇩

Secondary Antibody

binds to primary antibody

⇩

Wash

to remove excess antibody

⇩

Substrate (ECL)

substrate reacts with HRP bound to secondary antibody to create luminescent signal

⇩

Image

Detectlumine scent signal with film

Kit Contents

SuperLumia ECL Plus HRP Substrate Kit Contains sufficient substrate for 1200 or 2500 cm^2 of membrane surface. The following components are included:

Components	Size (EGB003S)	Size (EGB003M)
ECL Plus A	60 mL	250 mL
ECL Plus B	60 mL	250 mL

Storage

Store at 2-8 ℃.

Note:

① Optimize your western blot procedure for best results. Variables include sample amount, gel type, transfer method, membrane type, blocking reagent, wash buffer, primary and secondary antibody concentrations, and incubation times.

② Use a sufficient volume of all solutions to ensure that membranes do not dry out.
③ Use a shaking or rocking platform during incubation steps for optimal results.
④ Do not use sodium azide in buffers, because it inhibits HRP.
⑤ Always wear gloves or use clean, plastic forceps. Metallic devices (e.g., scissors) must have no visible signs of rust, which may cause speckling and /or high background.
⑥ The substrate Working Solution is stable for 1 hour at room temperature. Avoid exposure to the sun or other intense light. Short-term exposure to lab lighting is okay.

Usage

① Wash membrane
After protein transfer, remove the blot from the transfer apparatus and wash the membrane in deionized water for 5 minutes using agitation to remove all transfer buffer.
② Block membrane
Block nonspecific sites with Blocking Reagent)for 30–60 minutes at room temperature with shaking. Alternatively, block overnight at 2–8ºC without shaking.
③ Add primary antibody
Incubate the membrane with primary antibody solution (0.05 – 1 μ g/mL or follow manufacturer ' s recommended dilution) containing 10% blocking solution with continuous rocking for 1 hour. If desired, incubate the blot overnight at 2–8°C.
④ Wash membrane
a. Wash the membrane for 10 minutes using agitation with Tris-buffered saline (TBS), phosphate-buffered saline (PBS), or other physiological wash buffer containing 0.05% Tween 20 detergent.
b. Repeat wash step 2 more times.
c. Proceed to next step, or if using an enzyme-conjugated HRP primary antibody, proceed to Step 6.
⑤ Add secondary antibody
Incubate blot with the secondary antibody HRP-conjugate working dilution (0.005–0.04 μg/mL or 1:25,000–1:200,000, from a 1 mg/mL stock solution) for 30 minutes to 1 hour at room temperature using shaking.
⑥ Wash membrane
Wash the membrane 6 times for 5 minutes each in wash buffer to remove any unbound secondary antibody conjugate. It is crucial to thoroughly wash the membrane after incubation with the HRP enzyme conjugate.
⑦ Prepare substrate
Prepare the substrate working solution by mixing ECL Plus A and ECL Plus B at a 1:1 ratio, use 0.1 mL working solution per cm2 of membrane.
Note: The working solution is stable for up to 1 hour at room temperature.
⑧ Develop substrate
Incubate the membrane with the substrate working solution for 5 minutes.
⑨ Image membrane
a. Remove blot from working solution and place it in a plastic sheet protector or clear plastic wrap.
b. Use an absorbent tissue to remove excess liquid and carefully press out any bubbles from between the blot and the membrane protector.
c. Image the blot using an imaging system or X-ray film.