Enhanced Chemiluminescent Reagent Kit (Anti-Rabbit IgG)

Catalog No. EK1002

Description Enhanced Chemiluminescent Reagent Kit (Anti-Rabbit IgG) is a four-component system for sensitive detection of membrane-immobilized proteins on western blots probed with rabbit primary antibodies using HRP-conjugated anti-rabbit secondary antibodies and enhanced chemiluminescent peroxidase substrate reagents.

Application Western blot

Pack Size 1 kit (for 1000 cm² membrane)

Reagent Type Protein assay kit; ECL Western Blotting related

Storage 4°C for one year.

Introduction Enhanced Chemiluminescent Reagent Kit (Anti-Rabbit IgG) is a four-component system for sensitive detection of membrane-immobilized proteins on western blots probed with rabbit primary antibodies using HRP-conjugated anti-rabbit secondary antibodies and enhanced chemiluminescent peroxidase substrate reagents.

Kit Components

1. Blocking buffer: 10 g protein dry powder (nonfat dry milk). Make the blocking buffer by dissolving 2 g protein dry powder in 100 ml Diluent buffer.
2. HRP-conjugated goat anti-rabbit IgG: Affinity purified antibody.
3. Enhanced chemiluminescent chromogenic reagent, containing:
   - Enhanced chemiluminescent (ECL) reagent A: 5 ml. 20×.
   - Enhanced chemiluminescent (ECL) reagent B: 5 ml. 20×.
   (Volume is sufficient for covering up to 1000 cm² of membrane.)

Material Required But Not Provided

- Nitrocellulose or PVDF membrane.
- Diluent Buffer (for preparation of blocking buffer and antibody solution): Add 2.42 g Tris, 9 g NaCl, 850-900 μl pure acetic acid into 1000 ml distilled water, adjust pH to 7.2-7.6.
- Wash Buffer: Add 0.5 ml of TWEEN 20 into 1000 ml of diluent buffer.
- Primary antibody. This kit applies to the primary antibodies raised from rabbit.
Main Advantages

Specific
High signal-to-noise ratio

Sensitive
Up to 10 times more sensitive than colorimetric methods

Quantitative
Linear response in the range 0.2 to 2 OD units

Stable
The light output signal remains detectable up to several hours after incubation

Reliable
Possibility for repeated exposure to X-ray film until obtain optimal results

Storable
Immediate hard copy data - avoid fading of results

Reuseable
Allowing reprobing on membranes up to 10 times - saves time and materials

Fast
Short reaction times; few sec to few min of exposure enough to detect signal

Non-radioactive and Non-toxic
No specialized facilities needed

Assay Information

Sample Type
SDS-PAGE separated-, membrane-immobilized-, primary-antibody-probed proteins from cell/tissue lysates

Assay Type
Immonoassay

Assay Purpose
Detect target protein; Quantify target protein level

Technique
Immunodetection of target protein by HRP-conjugated secondary antibody

Detection Method
Chemiluminescent

Emission
428 nm, blue glow

Equipment Needed
WB instrumentation; X-ray film cassette or a charge-coupled device (CCD) imager

Assay Sensitivity
Femtomole and picogram protein quantities

Background
Chemiluminescence refers to the light emitted by a chemical reaction and chemiluminescent systems have emerged as the best all-around technique for the revelation step of blotting-based analyses. Horseradish Peroxidase (HRP) Conjugated Secondary Antibodies are the most popular antibody conjugates and are widely used to obtain chemiluminescent results in western and other immunoblotting, ELISA, and immunochemistry. In the chemiluminescence reaction Horseradish peroxidase (HRP) catalyzes the oxidation of cyclic diacylhydrazides, such as luminol, or acridine derivatives in the presence of hydrogen peroxide.
(H2O2) acting as activator or oxidizing agent. Immediately following the oxidation, the luminol is in an excited state (intermediate reaction product), which decays to the ground state by emitting light. Strong up to 1000-fold enhancement of the light emission is produced by enhancers, such as phenolic compounds, making the light emission much prolonged and very intense. This allows detection of protein at a level of 1-10 pg.

Enhanced Chemiluminescent (ECL) Reagent Kit is an immunodetection system that utilizes horseradish peroxidase (HRP) conjugated anti-mouse, anti-rabbit, anti-goat, or anti-rat secondary antibodies for ECL western blot detection of proteins. Proteins from various samples such as cell lysates are first separated within an acrylamide gel under adapted electric current (electrophoresis) and then transferred onto a PVDF or nitrocellulose membrane (transfer). The membrane containing the proteins is then incubated with specific primary antibodies which bind to their specific target(s) (capture) and subsequently with Horseradish Peroxidase (HRP)-conjugated secondary antibodies which bind to the primary antibodies (detection). Addition of chemiluminescent reagents (HRP luminol-based substrate, enhancer and activator) results in a chemiluminescent signal that can be captured using photographic or other imaging methods (revelation). The peroxidase-catalyzed oxidation of luminol produces a glow-type light emission at 428 nm. The intensity of light output is proportional to peroxidase activity and is a measure of the number of enzyme molecules reacting and thus of the amount of target antigen.

Assay Procedure
1. Run protein sample and molecular weight standard through polyacrylamide gel electrophoresis (PAGE).
2. Transfer the protein sample to a nitrocellulose membrane or PVDF membrane.
3. Block membrane: Immerse the membrane in blocking buffer and incubate at 20-30°C for 1.5-2 hours or at 4°C overnight with agitation.
4. Wash membrane once for 10 minutes in Wash Buffer.
5. Incubate membrane with primary antibody: Dilute primary antibody in Diluent Buffer. Incubate membrane with primary antibody solution at 20-30°C for 2 hours or at 4°C overnight with agitation. Follow the antibody manufacturer’s recommendations for optimized concentration.
6. Wash membrane in Wash Buffer with gentle agitation, 3 times for 10 minutes each.
7. Incubate the membrane with diluted secondary antibody at 20-30°C for 90 minutes or at 4°C overnight. Secondary antibody dilutions typically range from 1:2000-1:10000. Optimal secondary antibody dilutions must be determined empirically.
8. Wash membrane in Wash Buffer with gentle agitation, 4 times for 5 minutes each.
9. Chemiluminescent Detection: Add 50μl chromogenic reagent A and 50μl chromogenic reagent B into 1 ml of distilled water and mix well. Add the working solution onto the membrane and incubate at room temperature until bands appear (usually 30 seconds-5 minutes). Note: It is better to prepare the working solution just before use.