BOSTER
Antibody and ELISA Experts

BOSTER BIOLOGICAL TECHNOLOGY Co.,Ltd.
3942 B Valley Ave, Pleasanton, CA, 94566

Phone: 888-466-3604 Fax: 925-215-2184 Email: boster@bosterbio.com Web: www.bosterbio.com

Enhanced Chemiluminescent Reagent Kit (Anti-Mouse IgG)

Catalog No. EK1001

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

Application Western blot

Pack Size 1 kit (for 1000 cm^2 membrane)

Reagent Type Protein assay kit; ECL Western Blotting related

Storage 4°C for one year.

Introduction

Enhanced Chemiluminescent Reagent Kit (Anti-Mouse IgG) is a four-component system for sensitive detection of membrane-immobilized proteins on western blots probed with mouse primary antibodies using HRP-conjugated anti-mouse 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-mouse 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 cm^2 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 mouse.

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	Immunoassay
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.