BOSTER
Antibody and ELISA Experts

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Western Blotting Goat IgG DAB Chromogenic Reagent Kit (Yellow)

Catalog No. SA2021

Size 1 kit (1200 cm^2)

Storage -20°C for one year. DAB reagent should be protected from light.

Introduction

DAB is the chromogenic substrate of peroxidase. The reaction product is a brown precipitate insoluble in water, dimethylbenzene or alcohol, which makes DAB suitable for color development reaction in western blotting. Boster DAB Chromogenic Reagent kit is extremly senstive and has a high signal-to-noise ratio.

Kit Components

1. Blocking buffer: 2×10 g protein dry powder.
   Make the blocking buffer by dissolving 2 g protein dry powder in 100 ml Diluent buffer.
3. HRP-conjugated rabbit anti-goat IgG: 0.2 ml.
4. DAB chromogenic reagent, containing:
   - Chromogenic reagent A: DAB concentrated solution, 3 ml. 40×.
   - Chromogenic reagent B: H_2O_2 concentrated solution, 3 ml. 40×.
   - Chromogenic reagent C: TBS concentrated buffer, 3 ml. 40×.

(Volume is sufficient for covering up to 1200 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 goat.

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, B and C into 2 ml of distilled water and mix well. Add the working solution onto the membrane and incubate at room temperature until bands appear (usually 1-5 minutes). Wash the membrane with distilled water to stop the reaction.
10. Observe the bands and take pictures.

Note

- Chromogenic reagent A should be stored at –20°C. If crystal appears, fully dissolve it before use.
- Chromogenic working solution should be freshly prepared.