Product Information Sheet

Western Blotting Rat IgG DAB Chromogenic Kit (Yellow)

Catalog No. SA2023

Size 1 kit

Storage - 20°C for 1 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.

This Western Blotting DAB Chromogenic kit provides an easy, consistent, sensitive and highly specific detection solution to your protein of interest. Our proprietary polymeric labeling of peroxidase conjugated secondary antibodies offers superior sensitivity in western blotting, significant increase in the detection range, high signal - to - noise ratio, and lower primary antibodies consumption. Powered by our proprietary polymeric labeling technique, DAB substrates, a unique blocking agent, highly specific secondary antibodies and carefully optimized protocols, this kit yields cleanest results, blot after blot. For a more complete guide, please see the assay procedure section.

Kit Components

1. Blocking Reagent: 2×10g protein dry powder.
2. Concentrated antibody diluent solution: 20 ml/bottle. 10×.
3. Polymeric peroxidase - conjugated rabbit anti - rat IgG: 0.2 ml. 200 - 400×.
4. DAB chromogenic reagent, containing:
    - Chromogenic reagent A: DAB concentrated solution, 3ml. 40×.
    - Chromogenic reagent B: H_2O_2 concentrated solution, 3ml. 40×.
    - Chromogenic reagent C: TBS concentrated buffer, 3ml. 40×.

Material Required But Not Provided

- Nitrocellulose or PVDF membrane.
- Diluent Buffer (for preparation of blocking reagent and antibody dilutions): Add 2.42g Tris, 9g NaCl, 850- 900µl pure acetic acid into 1000ml 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: User provided. This kit applies to the primary antibodies raised from rat.

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 the membrane: make the blocking solution by dissolving 2g protein dry powder (Component 1) in 100 ml Diluent Buffer; completely submerge the membrane in the blocking solution and incubate at 20- 37°C for 1- 2 hours.
4. Wash membrane once for 5 minutes with Wash Buffer.
5. Preparation of Antibody Diluent Solution: add 1g protein dry powder and 10ml of concentrated antibody diluent solution (Component 2) into 90ml of Diluent Buffer. Dilute primary antibody (user‐supplied) and secondary antibody (Component 3) with the Antibody Diluent Solution.
6. Incubate the membrane in diluted primary antibody at 20‐37°C for 2 hours. Follow the antibody manufacturer's recommendations for best concentration. In case of absence of the specific bands or weak positive staining, increase the concentration of the primary antibody; in case of presence of non‐specific bands, decrease the concentration of the primary antibody.
7. Wash the membrane by agitating it in Wash Buffer, 3 times for 5 minutes each.
8. Incubate the membrane with diluted secondary antibody at 37°C for 90 minutes. The user may adjust the dilution based on actual staining.
9. Wash the membrane by agitating it in Wash Buffer, 4 times for 5 minutes each.
10. Use DAB chromogenic reagent to detect the bands. Add one drop of each chromogenic reagent A, B and C (Component 4) into 2ml of distilled water and mix thoroughly. Add the resulting solution to the membrane and develop at room temperature until bands appear (usually 1‐30 minutes). Wash the membrane with distilled water to stop the reaction.
11. Observe the bands and take pictures.

Note

- "Rat IgG" refers to the origin of animal species of the primary antibody, not the origin of the specimen. This kit applies to the primary antibodies raised from rat.
- Store the kit at 4°C for frequent use, or at ‐20°C for infrequent use. The shelf life at ‐20°C is one year.
- Chromogenic reagent A should be stored at ‐20°C. If crystal appears, fully dissolve it before use.
- Chromogenic working solution should be freshly prepared.

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