Protocol

Western Blotting Protocol

utilizing NBT

Introduction

Immunoblotting (also called Western Blotting) is used extensively in different protein studies aiming to characterize specific proteins, their interactions, and their modifications. This assay allows the detection of specific antigens by recognizing them with polyclonal or monoclonal antibodies. First, protein samples are solubilized, the antigen is separated by SDS-PAGE and transferred to a membrane, which is then probed with primary and secondary antibodies. Finally, antibody-antigen complexes are identified with chromogenic or luminescent substrates. One commonly-used chromogenic substrate is nitrotetrazolium blue chloride (NBT). NBT, when used in the presence of alkaline phosphatase and in combination with 5-bromo-4-chloro-3'-indolyphosphate p-toluidine (BCIP), causes an oxidation reaction that results in the creation of a purple-brown color. Here, we describe a general procedure using NBT and BCIP to visualize proteins on a blot.

Materials

- NaCl
- Tris (GoldBio Catalog # T-400)
- $MgCl_2$
- HCl
- NBT (GoldBio Catalog # NBT)
- Wash buffer- TBST or TBS
- BCIP (GoldBio Catalog # B-500)
- Alkaline phosphatase conjugate
- Molecular biology grade water

Method

1. Prepare a substrate buffer by adding 100mM NaCl, 0.1M Tris and 5mM $MgCl_2$ to a final pH of 9.5. To adjust the pH, add hydrochloric acid.

2. Prepare a stock solution of NBT at 10 mg/ml, and a BCIP stock solution at 50 mg/ml, both in molecular biology grade water.

3. Add 33 µl of 50 mg/ml of BCIP stock solution and 330 µl of 10 mg/ml NBT stock solution to 10 ml of substrate buffer.

4. Calculate the volume of primary antibody to use. Dilute primary antibody in blocking buffer. After incubation with primary antibody, incubate the blot with an alkaline-phosphatase secondary antibody conjugate in wash buffer (TBST or TBS).

Note: Browse GoldBio antibody collection for a large variety of primary antibodies for your research. For all GoldBio primary antibodies, use our convenient Antibody Dilution Tool to calculate the volume you need.

5. Wash the blot with wash buffer (TBST or TBS), add the NBT/BCIP solution, ensuring the blot is covered with reagent during color development.

6. Incubate the blot at room temperature with the reagent for 10 minutes.

Note: The blots and/or procedure may affect the time needed for color development.

7. In order to stop color development, rinse the blot with molecular biology grade water.

Associated Products

- Tris (GoldBio Catalog # T-400)
- NBT (GoldBio Catalog # NBT)
- BCIP (GoldBio Catalog # B-500)

References

Blake, M., Johnston, K., Russell-Jones, G., & Gotschlich, E. (1984). A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Analytical Biochemistry, 136*(1), 175-179. Doi:10.1016/0003-2697(84)90320-8.