Standard Protocol for Western Blotting

1. Prepare a substrate buffer by adding 100mM NaCl, 0.1M Tris and 5 mM $MgCl_2$ to a final pH of 9.5. To adjust the pH, add hydrochloric acid.
2. Prepare Nitrotetrazolium blue chloride (NBT) (GoldBio Catalog # NBT) stock solution 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. Rinse specimens with an alkaline phosphatase conjugate in a wash buffer before adding the NBT/BCIP solutions and cover specimen with the reagent during color development.
5. Keep the specimen at room temperature with the reagent for 10 minutes. Note: The specimens and/or the procedure may affect the time needed for color development.
6. In order to stop color development, rinse specimen with molecular biology grade water.

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