

BCIP-NBT Solution Kit for Alkaline Phosphatase Stain

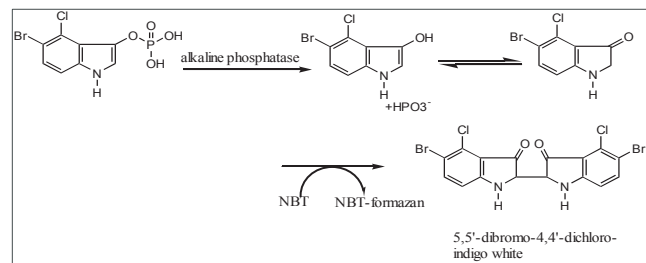
Detection System for Western Blotting

Features

- Highly sensitive alkaline phosphatase (ALP) staining kit for blotting.
- Buffer contains ALP catalyst for the same detection sensitivity of conventional methods. Only half the reaction time of conventional methods.
- Stable: coloring substrate BCIP and color coupler NBT does not react in the supplied mixture.
- Easy preparation: ready to use ; the kit contains all reagents.
- Nitroblue Tetrazolium (NBT), as a color coupler, for clear spots or clear bands.
- This product is DNase and RNase free.

Easy to use: BCIP and NBT are premixed
Fast: half the reaction time of conventional kits

Principle



BCIP-NBT reaction scheme.

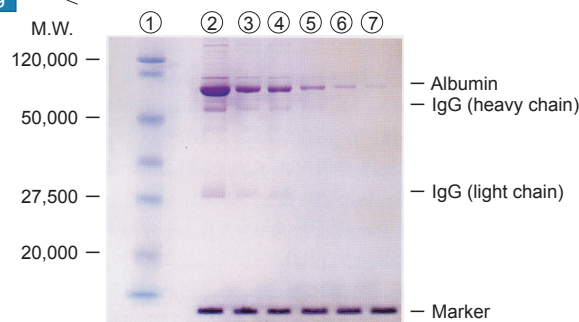
BCIP is hydrolyzed by alkaline phosphatase to form a blue intermediate. The intermediate is then oxidized by NBT to produce a dimer, which is an intense insoluble purple dye.

Application

Western blotting :

After blotting antigen conjugates to the antibody-ALP in Western blotting, the targeted protein is detected with high sensitivity. In the following experiment protein as little as 0.2 µg was detected.

CBB staining



< Protocol >

Separate protein by electrophoresis

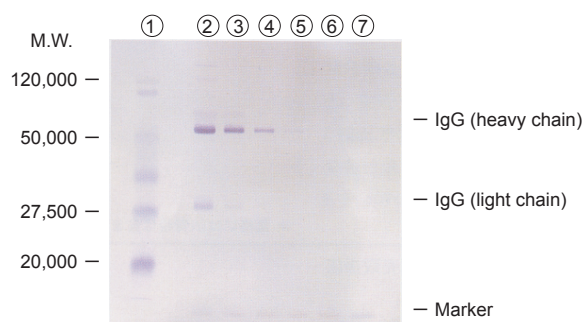
Transfer to membrane

Blocking

Reaction with primary antibody

Wash

Staining reaction



< Conditions >

Sample : human serum
Sample amount : ① prestained markers
② 5 µg ③ 1.7 µg ④ 0.55 µg
⑤ 0.2 µg ⑥ 60 ng ⑦ 20 ng

Primary antibody : Anti-human IgG (Goat)
Secondary antibody : Anti-goat IgG (Rabbit) ALP conjugated

Electrophoresis : 12.5% SDS-PAGE (35 mA, 40 minutes)
Membrane : PVDF membrane
Exposure time : 30 minutes

Composition

Content	Components	PKG Size	Storage
Staining Stock Solution	5-Bromo-4-chloro-3-indolyl Phosphate (BCIP) / Nitroblue Tetrazolium (NBT)	2 ml x1	-20 °C
Buffer Solution	Tris-HCl buffer / Magnesium Chloride	200 ml x 1	4 °C

Preparation

Prepare staining solution freshly prior to use, and start the reaction within 10 minutes. To leave the prepared staining solution for 30 minutes or more may result in false result.

Preparation of staining solution (for membrane size of 10 cm x 10 cm)

- (1) Shake and mix Buffer solution thoroughly.
- (2) Pour 50 ml of Buffer solution into a 50 - 100 ml measuring cylinder. Add 0.5 ml of Staining stock solution using micropipette, and mix thoroughly. For a different volume, simply dilute the Staining solution by 100 times of the volume of the Buffer solution.

Protocol

1. Wash the membrane (with bound alkaline phosphatase labeled antibody after Western or dot blotting) with Tris buffered saline (TBS) containing appropriate surfactant (i.e. 0.05% Tween 20 etc).

2. Add the freshly prepared staining solution into a clean plastic tray. Immerse the washed membrane into the prepared staining solution. Let stand or shake gently at room temperature.

3. When the expected band appears on the membrane, remove membrane from the staining solution. Wash with running water for 10 minutes or longer to stop the staining reaction.

Reference

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Attention

For rinsing the membrane after antibody-ALP conjugation, use Tris buffered saline (TBS) containing surfactant (i.e. 0.05% Tween 20). Alkaline phosphatase may react with phosphoric acid and may result in loss of enzyme activity if PBS is used for washing the membrane.

Caution

- To avoid contamination, carefully wash the measuring cylinder and the plastic tray with water, then in 1M sulfuric acid solution for at least 30 minutes, and finally with deionized water before drying. Contaminated equipment causes false result by reacting with the staining solution.
- The staining solution changes color gradually with time as a result of reduction. Prepare the staining solution freshly prior to use, and start reaction within 10 minutes. To leave the prepared reagent for 30 minutes or more may result in false result.
- If the concentration of alkaline phosphatase labeled antibody is too high, strong background will appear on the membrane. Optimize the concentration of alkaline phosphatase labeled antibody for best result.
- If the rinsing of membrane after staining is not thorough enough, the whole membrane may become dark after drying. Rinse the membrane fully after staining.
- The Staining stock solution has toxic and irritant substances. If it accidentally comes into contact with skin, wash the skin with copious amount of water.
- Store the product strictly according to the recommended storage condition.
- Wear protective gloves when handling this product.

Expiration

One year from manufacturing. Expiration date is stated on the product label (Exp. yy / mm)

Ordering Information

Product name	Grade	Storage	Code No.	PKG Size
BCIP-NBT Solution Kit for Alkaline Phosphatase Stain	SP	F	03937-60	1 kit

1 kit (to make approximately 200 ml staining solution)

For research use only, not intended for diagnostic or drug use.

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