BIO BASIC
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Product information

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INT/BCIP Stain Kit

Catalog #: PW033
Size: 5 preps
Storage: 2~8°C

Product Description:

INT refers to 2-[4-Iodophenyl]-3-[4-nitrophenyl]-5-phenyl-tetrazolium chloride, while BCIP stands for 5-bromo-4-chloro-3-indolyl-phosphate. When exposed to alkaline phosphatase (AP), BCIP undergoes hydrolysis, generating reactive compounds that interact with INT, leading to the formation of observable reddish or saffron-yellow precipitates. The INT/BCIP Stain Kit is commonly utilized for a variety of purposes, including immunochemistry, Western blotting, in situ hybridization, or alkaline phosphatase color reactions in cells or tissues.

Composition:

This kit is suitable for Dot Blot, Western Blot, and In Situ hybridization. It consists of Solution A, Solution B, Reaction Buffer, and Stop Buffer, which allows for approximately five applications. Here are the component quantities:

Components	
Solution A	0.62ml
Solution	0.62ml
Reaction Buffer (5X)	10ml
Stop Buffer	50ml

Storage and Transportation:

Transport at controlled room temperature. Store solutions at 2-8°C, shielded from light.

Procedure:

1. In Western blot procedures, after the membrane has been incubated with the appropriate AP secondary antibody at 37°C with continuous rotation for more than 30 minutes, conduct a 5-minute wash step using TBST or PBST wash buffer, repeat this wash step 3-4 times. Conclude with a final wash using TBS or PBS, and store the membrane in a dark environment.

2. Before initiating the chromogenic reaction, transfer 10ml of the diluted Reaction Buffer, along with 0.12ml of Solution A and 0.12ml of Solution B, into a clean 15ml brown bottle. Thoroughly vortex the mixture and retain it for subsequent steps.

3. In a dark area, transfer the 10ml mixed solution from the previous step onto the membrane-containing container. Place the container on a rotator and incubate this at 37°C for approximately 30 minutes. This rotational incubation will lead to the formation of reddish or saffron-yellow insoluble material on the membrane.

4. Next, immerse the membrane in 10ml of Stop Buffer and vortex for 1 minute. Subsequently, rinse the membrane with DDH^2O 3-4 times.

5. For optimal results, capture images of the blot within an hour for analysis, as delaying the process may lead to color fading.

Notices

1. To prevent excessive color development and a high noise-to-signal ratio, control the color reaction time to around 30 minutes.

2. After use, securely close the lids of the Reaction Buffer, Solution A, and Solution B bottles. Additionally, wear gloves and appropriate clothing to avoid contact with the skin. Dilute the 5x concentrated Reaction Buffer with DDH^2O.

3. It is recommended to use a 6x8cm-sized membrane. If a larger membrane is used, adjust the quantity of reagents accordingly to ensure complete immersion of the membrane.

4. Ensure the correct preparation of reagents for Western blotting, membrane transfer, antibody dilution, and the washing procedure to minimize noise and achieve accurate color reactions.

5. With the exception of Solution, A and Solution B, which must be stored at 2-8°C and protected from light, the other reagents can be stored at low temperatures. The kit has a one-year expiration period.

6. The complete color reaction solution should be prepared immediately, and any remaining solution should be discarded.