PRODUCT INFORMATION

NBT/BCIP Stain Kit

Product information for PW032:

Introductions

NBT and BCIP refers to Nitro-Blue-Tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate respectively. When mixed together, BCIP is hydrolyzed and reacts with NBT, which then react with O_2, forming visible purple precipitates in the presence of AP (alkaline phosphatase).The kit can be used for Western Blot, Dot Blot and In Situ Hybridization.

Compositions:

1. Solution A	0.21 ml
2. Solution B	0.51 ml
3. Reaction Buffer	10ml (5X)
4. Stop Buffer	50 ml

Storage Condition

Transportation at room temperature. Upon receipts, store Solution A and Solution B at 2-8 ^{o}C and avoid light. Store Reaction buffer and Solution C at 2-8^{o}C.

Procedures

1. For western blot experiments, immerse membrane in secondary antibody solution and incubate at 37^{o}C on a rotator for more than one half an hour. Wash the membrane with TBST or BPST wash buffer for 5 minutes and repeat the wash step for 3-4 times. Keep the membrane in dark.
2. Before carrying out chromogenic reaction, pipette **8ml** diluted Reaction Buffer, **0.04ml** Solution A and **100ul** Solution B into a clean 15ml brown bottle. Vortex to mix.
3. In a dark place, transfer the above 10ml mixed solution into the container that have the membrane, incubate at 37^{o}C and shake on a rotator for about half an hour, purple insoluble matter will appear on membrane. Avoid from light.
4. Add 10ml Stop Buffer and vortex for 1 minutes ,then wash the membrane with DDH_2O for 3-4 times.
5. Take photo in an hour and analyze the result before the color fades away.

Notices:

1. Control colour reaction time in 30 minutes or less. Otherwise high noise-signal ratio may develop.
2. After usage, please tighten the lid of Reaction Buffer, Solution A and Solution. Wear glove at all times.
3. Recommend to use 6x8 cm size membrane. If a larger membrane, please adjust the volume of reagents proportionally to completely immerse the membrane.
4. Solution A and Solution B must store at 2-8^{o}C and avoid light. Other reagents can store at low temperature and have one year expiration.
5. The reaction solution must prepare fresh, and left-over working stock must be discarded.

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