Product Information

SIGMA*FAST*™ BCIP®/NBT Tablet

B5655

Product Description

5-bromo-4-chloro-3-indolyl phosphate (BCIP®) and nitro blue tetrazolium (NBT) are reagents that are widely used in tandem for detection of alkaline phosphatase conjugates.[1-3] SIGMA*FAST*™ BCIP®/NBT tablets are useful in immunochemistry to detect alkaline phosphatase. Common uses are in Western blotting or dot blotting, and to a lesser extent in immunohistology.

SIGMA*FAST*™ BCIP®/NBT tablets require no additional buffers or steps to prepare an active substrate solution. One tablet, dissolved in 10 mL of water, provides 10 mL of ready-to-use buffered substrate solution. The substrate solution contains:

- BCIP (0.15 mg/mL)
- NBT (0.30 mg/mL)
- Tris buffer (100 mM)
- $MgCl_2$ (5 mM)

The solution pH is in the range of 9.25 – 9.75.

Several theses[4-6] and dissertations[7-16] have cited use of product B5655 in their protocols.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the tablets at –20 °C.

Preparation Instructions

- Remove the tablet package from the freezer. Allow it to warm to room temperature.
- Open the foil pack. Drop the SIGMA*FAST*™ BCIP®/NBT tablet into an appropriate container. **Do not touch the tablet with your fingers**.
- Add 10 mL of water. Vortex until dissolved (2-5 minutes).

The SIGMA*FAST*™ BCIP®/NBT Substrate Solution is now ready for use. For best results, the Substrate Solution should be used within one hour.

Procedure

1. Pour the SIGMA*FAST*™ BCIP®/NBT Substrate Solution into a suitable container.
2. Lay the nitrocellulose paper in the solution. Make sure the paper is completely covered with the Substrate Solution.
3. Remove the nitrocellulose paper when sufficient color has developed (5-10 minutes).
4. Rinse in water. Although several blots can be developed in this manner, the potential for carryover from blot to blot does exist and should be evaluated carefully.
5. Blots stained with SIGMA*FAST*™ BCIP®/NBT Substrate Solution may be dried and stored away from light for future reference.

Troubleshooting

Background is too high

- Use a blocking step prior to application of the primary antibody. Normal serum (10% v/v) from the same species as the second antibody generally produces the best results.
- Additional blocking agents for immunoblotting include:
  - 10% BSA
  - 0.05% TWEEN® 20
  - 3% nonfat-dried milk
    - Do not use milk as a blocking agent when using avidin-biotin systems.
- Decrease staining time.
- Titer the conjugate to optimize the working dilution.

No color develops or color is too faint

- Adjust the concentration of the primary antibody.
- Adjust the concentration of the secondary antibody.

- Determine if the enzyme conjugate is active.
- Consider using an amplifying system such as avidin-biotin.
- Increase the staining time.
- Adjust the transfer time of the samples to the nitrocellulose membrane.
- Increase the amount of sample.

References

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