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## Product Information

### SIGMA FAST™ BCIP/NBT Buffered Substrate Tablet

Product No. **B5655**

#### Use

SIGMA FAST BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium) tablets have been developed for use in immunochemistry as a precipitating substrate for the detection of alkaline phosphatase activity. 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 deionized or distilled water, provides 10 ml of ready-to-use buffered substrate solution.

Alkaline Phosphatase + BCIP → BCI + PO<sub>4</sub>, pH 9.5  
BCI + NBT → insoluble, blue color

#### Formulation

Each SIGMA FAST BCIP/NBT tablet contains the following when dissolved in 10 ml H<sub>2</sub>O:

BCIP	
(5-bromo-4-chloro-3-indolyl phosphate)	0.15 mg/ml
NBT (nitro blue tetrazolium)	0.30 mg/ml
Tris buffer	100 mM
MgCl <sub>2</sub>	5mM

**CAUTION** Refer to MSDS regarding cautions and handling instructions for this product.

#### Storage

Store at -20°C

#### Materials Included

SIGMA FAST BCIP/NBT tablets	5 or 25
Directions for use	1

#### Materials Required, not included

Distilled or deionized water  
Pipets or pipetors capable of delivering 10 ml  
Test tubes

#### Directions for Use

1. Remove the tablet package from the freezer and allow to warm to room temperature. Open the foil pack and drop the SIGMA FAST BCIP/NBT tablet into an appropriate container. **Do not touch the tablet with your fingers.** Add 10 ml of distilled or deionized water. Vortex until dissolved (approximately 2-5 minutes). SIGMA FAST BCIP/NBT substrate is now ready for use. For best results, solution should be used within one hour.
2. Pour SIGMA FAST BCIP/NBT substrate solution into a suitable container and lay the nitrocellulose paper in the solution. Make sure that the paper is completely covered with the substrate solution. Remove the nitrocellulose paper when sufficient color has developed (5 to 10 minutes). Rinse in distilled or deionized 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.
3. When finished, dispose of the remaining solution in a manner consistent with proper hazardous material handling protocols for your institution. Blots stained with SIGMA FAST BCIP/NBT may be dried and stored away from light for future reference.

#### Troubleshooting

1. Background is too high:
  - a. Use a blocking step prior to the application of the primary antibody. Normal serum (10% v/v) from the same species as the second antibody generally produces the best results.
  - b. Additional blocking agents for immunoblotting are 10% BSA, 0.05% Tween 20, or 3% Non-Fat Dried Milk .  
**Do not use milk as a blocking agent when using avidin-biotin systems.**
  - c. Decrease staining time.
  - d. Titer the conjugate to optimize working dilution.

2. No color develops or color is too faint:
  - a. Adjust the concentration of the primary antibody.
  - b. Adjust the concentration of the secondary antibody.
  - c. Determine if the enzyme conjugate is active.
  - d. Consider using an amplifying system such as avidin-biotin.
  - e. Increase the staining time.
  - f. Adjust the transfer time of the samples to the nitrocellulose membrane.
  - g. Increase the amount of sample.

#### References

1. Blake, M., Anal. Biochem., **136**, 175 (1984).
2. Horowitz, J., et al., J. Med. Chem., **9**, 447 (1966).

Pcs1/01

Please see reverse side of the invoice or packing slip.