Tribioscience

BCIP/NBT Substrate System (Catalog# TBS5022)

For colorimetric AP-based Blot & IHC detection

Catalog	Unit Size
TBS5022-125	125 mL
TBS5022-250	250 mL

DESCRIPTION

The 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) Substrate System is an ideal insoluble substrate for use with alkaline phosphatase. This system produces a blue-purple product. The intense color can be observed visually, is very stable, and will not fade upon exposure to light. The BCIP/NBT Substrate System offers a convenient, ready-to-use substrate solution for visualizing alkaline phosphatase activity in immunoblotting and immunohistology. Immunohistochemical staining with BCIP/NBT requires organic mounting media and can be counterstained with Nuclear Fast Red or Light Green. BCIP/NBT is generally a more sensitive method than Fast Red.

APPLICATION

BCIP/NBT Solution is formulated to precipitate and localize the blue-purple reaction product onto membrane surfaces or tissue samples at sites where the alkaline phosphatase-substrate reaction occurs. BCIP-NBT Substrate produces a very large signal-to-noise ratio, although its high sensitivity requires careful washing and blocking to avoid background staining. When performing immunohistochemical techniques, organic mounting media must be used.

KIT CONTENT

Size: 125 mL or 250 mL
Storage: Store at 4°C. Shelf life: 1 year.

DIRECTIONS FOR USE

Western blotting:

1. Remove blot from the transfer apparatus and block nonspecific sites with Blocking Buffer for 10-30 minutes at room temperature with shaking.
2. Add the primary antibody and incubate membrane for 1 hour with shaking.
3. Wash the membrane with TBS-T (TBS5008T).
4. Add the HRP-conjugated secondary antibody and incubate membrane for 1 hour at room temperature with shaking.
5. Wash membrane with TBS-T (TBS5008T).
6. Add the BCIP/NBT Substrate solution to the membrane and carefully monitor color development.
7. Stop the reaction by rinsing membrane with water.

Immunohistochemistry:

1. Rinse specimens incubated with an alkaline phosphatase conjugate in a non-phosphate wash buffer (TBS5008T) before treatment with the BCIP/NBT Substrate System.
2. Cover the entire specimen with the reagent during color development.
3. Incubate the specimen at room temperature with the BCIP/NBT reagent for ~10 minutes.
   Note: Specimens and procedure may affect the length of time needed for color development. Monitor color development to avoid over development. Stop color development by rinsing the specimen with water.

RELATIVE PRODUCTS

TBS2005	Protein Assay Kit
TBS5001	Protein Lysis Buffer
TBS5003-0	1xPBS
TBS5011-10	10 x PBST
TBS5008	TBS
TBS5008T	TBST
TBS5037	Urea Solution (8M)
TBS5038	Ammonium Sulfate Solution
TBS5040	0.5M EDTA Solution
TBS5041	MOPS Buffer
TBS5048	2% BSA in PBS
TBS5049-2	2% BSA in TBST
TBS5049-5	5% BSA in TBST
TBS5057	0.1% BSA ELISA Assay Buffer
TBS5077	Antigen retrieval Citrate Solution-pH6.0

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