INSTRUCTIONS

1-Step™ NBT/BCIP

34042

0560.2

Number	Description
34042	**1-Step NBT/BCIP,** 250mL

Storage: Upon receipt store product at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific 1-Step NBT/BCIP is a one-component sensitive precipitating alkaline phosphatase substrate for immunoblotting, immunohistochemistry, and *in situ* hybridization applications.

Important Product Information

- Mix the 1-Step NBT/BCIP bottle before use and then add the solution directly to the alkaline phosphatase until the desired color develops.
- To eliminate endogenous phosphatase activity, add a final concentration of 1mM levamisole to the 1-Step NBT/BCIP.

Procedure for Immunohistochemical Staining

1. Block nonspecific sites in the tissues with normal serum or other blocking buffer such as Thermo Scientific SuperBlock Blocking Buffer in TBS (Product No. 37535) for 30 minutes at room temperature in a humidity chamber. Drain the blocking buffer but do not remove excess.
2. Incubate tissues with primary antibody for 30 minutes at room temperature in a humidity chamber.
3. Wash tissues in Tris-buffered saline (TBS) for 3 minutes. Repeat wash step. Remove excess wash buffer.
4. Incubate tissues with alkaline phosphatase conjugated secondary antibody for 30 minutes at room temperature in a humidity chamber or with the ABC System.
5. Wash tissues with TBS for 3 minutes. Repeat wash step. Remove excess wash buffer.
6. Mix the 1-Step NBT/BCIP and add it to the tissue until desired stain develops.
7. Rinse slides with water.

Procedure for Western Blotting

1. Block membrane with a blocking buffer such as SuperBlock® Blocking Buffer in TBS (Product No. 37535) for 30 minutes at room temperature with shaking.
2. Incubate blot with the primary antibody for 1 hour with shaking.
3. Wash the membrane with Tris-buffered saline (TBS).
4. Incubate blot with alkaline phosphatase-conjugated secondary antibody for 1 hour at room temperature with shaking.
5. Wash membrane with TBS.
6. Mix the 1-Step NBT/BCIP and add it to the blot. Incubate blot for 5-15 minutes or until desired color develops.
7. Rinse blot with water.

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Procedure for *In Situ* Hybridization

Materials Required

- 50X FPG: 1% Ficoll® 400, 1% polyvinylpyrrolidone 360, 1% glycine
- 20X SSC Buffer: 150mM sodium chloride, 5mM sodium citrate; pH 7.0
- Hybridization Buffer: 50% deionized formamide, 5X SSC, 1.25X FPG, 31mM KH_2PO_4, 0.25% SDS, 31µg/mL salmon sperm DNA, 5% dextran sulfate
- TE Buffer: 10mM Tris•HCl, pH 7.6, 1mM EDTA
- Blocking Buffer: Blocker™ BSA in TBS (Product No. 37520) or SuperBlock Blocking Buffer in TBS (Product No. 37535)
- Alkaline Phosphatase-conjugated Streptavidin (Product No. 21324)

Method

1. Pre-treat slides with 1X FPG for 3 hours at 65°C to block nonspecific binding sites.
2. Pre-hybridize slides with Hybridization Buffer for 60 minutes.
3. Prepare 100-500ng/mL of biotinylated probe in Hybridization Buffer.
4. To detect DNA targets, incubate slide at 90°C for 4 minutes followed by quick cooling on ice for 4 minutes to denature probe and target DNA. To detect RNA targets, denature the DNA probe before applying to the slide.
5. Hybridize overnight at 45°C.
6. Wash slides with the following solutions with gentle agitation:
   - 2X SSC for 60 minutes
   - 1X SSC for 60 minutes
   - 0.2X SSC for 30 minutes
7. Add Blocking Buffer to the slide and incubate for 30-60 minutes at room temperature.
8. Add alkaline phosphatase-conjugated streptavidin and incubate for 1 hour at room temperature.
9. Wash slide with TBS.
10. Mix the 1-Step NBT/BCIP and add it to the tissue until the desired color develops. Stop development by rinsing slides in TE Buffer.

Related Thermo Scientific Products

31872	**Normal Goat Serum,** 2mL
31884	**Normal Rabbit Serum,** 2mL
31002	**NeutrAvidin® Protein, Alkaline Phosphatase Conjugated,** 2mg
21323	**Streptavidin, Alkaline Phosphatase Conjugated,** 3mg

General References

Altman, F.P. (1976). Tetrazolium salts and formazans. *Prog Histochem Cytochem* **9:**1-56.

Blake, M.S., *et al.* (1984). A rapid, sensitive method for detection of alkaline phosphatase-conjugated anti-antibody on Western blots. *Anal Biochem* **136:**175.

Evans, E.A., *et al.* (2003). SPAM1 (PH-20) protein and mRNA expression in the epididymides of humans and macaques: utilizing laser microdissection/RT-PCR. *Reprod Biol Endocrin* **1:**54.

Heller, S., *et al.* (1998). Molecular markers for cell types of the inner ear and candidate genes for hearing disorders. *Proc Natl Acad Sci USA* **95:**11400-5.

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Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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