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19880E_2010_0

Product No. 19880

BCIP-NBT Solution (Ready To Use)

BCIP-NBT Solution (Ready To Use) is used for the detection of alkaline phosphatase (AP) in immunoblotting and immunohistochemistry/immunocytochemistry (IHC / ICC) procedures. This product also can detect endogenous AP expressed in cell types such as iPS or osteoblast cells.

Features

- Available for wide range of applications
- Ready To Use solution
- DNase, RNase tested

Composition

5-Bromo-4-chloro-3-indoryl Phosphate (BCIP), Nitroblue Tetrazolium (NBT), enhancer, buffer

Protocol

For western blotting (dot blotting)

1. Wash the membrane probed with bound an AP-conjugated antibody after western or dot blotting with tTBS.
2. Pour the BCIP-NBT solution into a clean plastic tray. Immerse the washed membrane into the BCIP-NBT solution and incubate room temperature.
3. When band(s) of the expected size appear on the membrane, remove the solution and wash with water to stop the staining reaction. To store the membrane, put the dried membrane in a plastic bag, protected from light.

For IHC / ICC / in situ hybridization (ISH)

1. Wash the tissue or cells probed with AP-labeled antibodies with tTBS.
2. Add enough BCIP-NBT solution to cover the sample and let stand at room temperature.
3. At the desired staining intensity, remove the solution and wash with water to stop the staining reaction.
4. Mount the sample with aqueous mounting media[1)] or non- aqueous mounting media[2)].

[1)] Because diffusion may occur with use of certain types of aqueous mounting media, it is recommended to image the sample as soon as possible.
[2)] Do NOT dehydrate the sample with alcohol, which may reduce staining.

For staining endogenous AP

1. Fix the cells using a fixative reagent such as paraformaldehyde.
2. Wash the cells with water.
3. Add BCIP-NBT solution to the cells and let stand at room temperature. (Required volume is 1 mL for a 35 mm Dish)
4. At the desired staining intensity, remove the solution and wash with water to stop the staining reaction.
5. Mount or dry the sample, and image by microscopy.

Attention

It is not recommended to use PBS as the wash buffer, since AP may react with phosphoric acid to reduce enzymatic phosphatase staining.

Caution

- With weak signals, please prolong the incubation time. Should incubation overnight be required, please use a sufficient volume of solution to keep the sample from drying up.
- If the concentration of the AP-labeled antibody is too high, a strong background signal will appear. Optimize the concentration of AP-labeled antibody for best results.
- Background signals will appear with insufficient washing. Wash the sample thoroughly after staining.

Storage

Refrigerator

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Expiration

Expiration date is stated on the product label.

Packing

100 mL (Product No. 19880-84)

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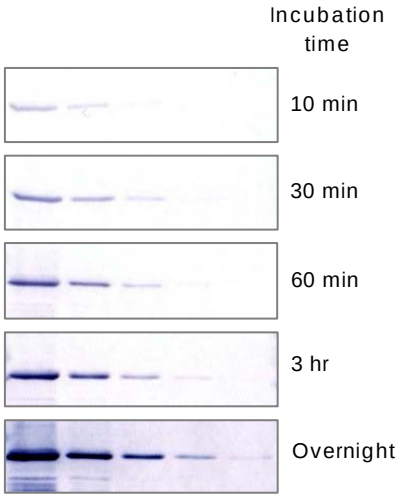
Reference Data

<Reactivity and Reaction time>
The extension of incubation time increase sensitivity.

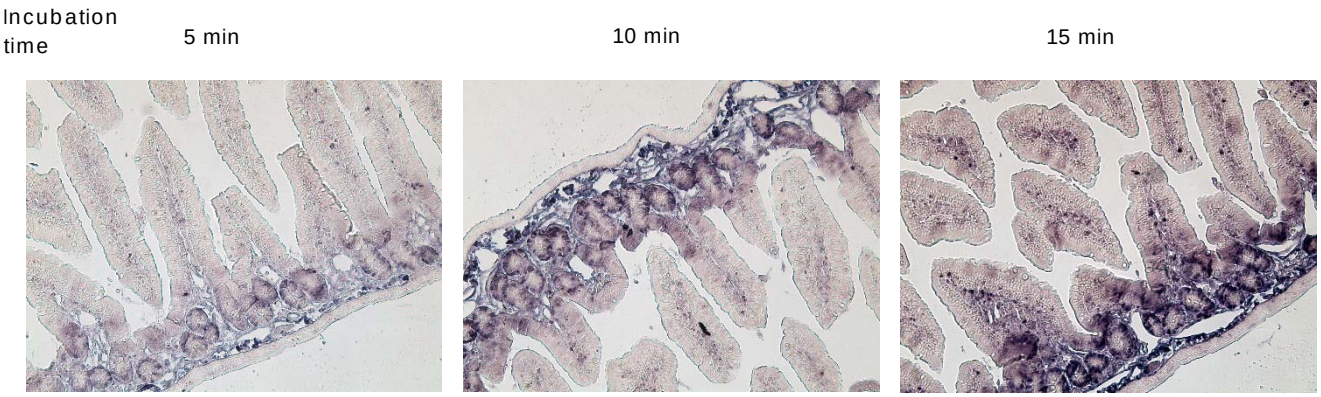
Application of Western Blotting

Sample	:	4 µg of HeLa cell lysate, 5 serial four-fold dilution series
Blocking	:	Bullet Blocking One (Product No.13779) 5 min at RT
1st antibody	:	GAPDH Antibody (Novus #NB300-322) 1:20,000 Diluted with Bullet ImmunoReaction Buffer(Product No.18439) 30 min at RT
2nd antibody	:	Goat anti-Rabbit IgG (H+L) Secondary Antibody [Alkaline Phosphatase] (Novus #NB7181) 1:100,000 Diluted with Bullet ImmunoReaction Buffer (Product No.18439) 30 min at RT
Detection	:	BCIP-NBT Solution (Ready To Use)



Application of immunohistochemistry

Sample	:	Mouse small intestine
Antigen retrieval	:	Histo VT One (Product No. 06380) 20 min at 90℃
Blocking	:	Blocking One Histo (Product No. 06349) 10 min at RT
1st antibody	:	PCNA Antibody (Novus #NB100-456) 1:500 overnight at 4℃
2nd antibody	:	Goat anti-Rabbit IgG (H+L) Secondary Antibody [Alkaline Phosphatase] (Novus #NB7181) 1 hr at RT
Detection	:	BCIP-NBT Solution (Ready To Use)
Mounting media	:	CC/Mount™ (Diagnostic BioSystems #K002)
Microscopy	:	Olympus BX-50 (X25)



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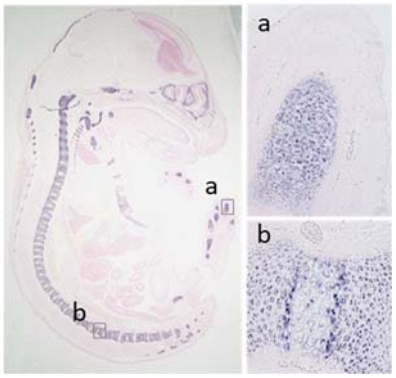
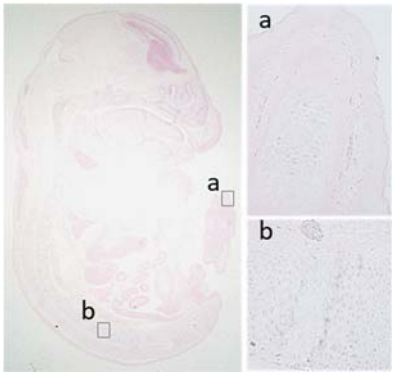
<Applications>

Application of ISH (mRNA of collagen type IX(a1) on mouse embryo)

Sample	:	mouse embryo E16.5
Hybridization	:	DIG labeled mouse CoL9a1 RNA probe for anti-sense(left) or sense (right) overnight at 60℃
Blocking	:	Blocking One Histo (Product No. 06349) 10 min at RT
1st antibody	:	Sheep Anti-DIG, Alkaline Phosphatase(AP) Conjugated（Roche #11093274910）
Detection	:	BCIP-NBT Solution（Ready To Use） 4 hr at RT
Counter stain	:	Kernechtrot 10 sec at RT
Microscopy	:	Asone 1-1925-02(X2.6), Olympus BX-50 (X25)



Anti-sense



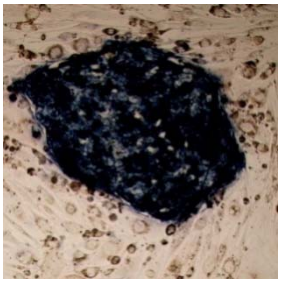
Sense

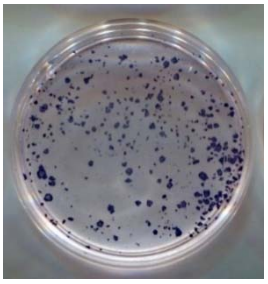
Application of endogenous AP staining (iPS cell)

Sample	:	iPS cell (201B7*), Feeder cells (SNL)
Fixation	:	4%-Paraformaldehyde Phosphate Buffer Solution (Product No. 09154) 10 min at RT
Detection	:	BCIP-NBT Solution（Ready To Use） 1 hr at RT

*Takahashi, K. et al. Cell, 2007 Nov 30;131(5):861-872

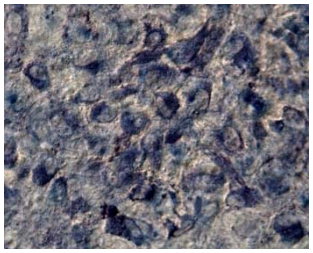
(left) Cover the sample with a coverslip without mounting media and observe using Olympus BX-50)(X10)
(right) Dry the sample and scan it.


Microscopy image


Scan image

Application of endogenous AP staining (osteoblast cells)

Sample	:	MC3T3-E1 cell Cells are cultured with (left) or without (right) 100 ng/mL BMP-2 (Pepro Tech #120-02) for 3 weeks.
Fixation	:	4%-Paraformaldehyde Phosphate Buffer Solution (Product No. 09154) 10 min at RT
Detection	:	BCIP-NBT Solution（Ready To Use） 30 min at RT
Microscopy	:	Olympus BX-50 (X50; DIC)


With BMP-2


Without BMP-2

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