



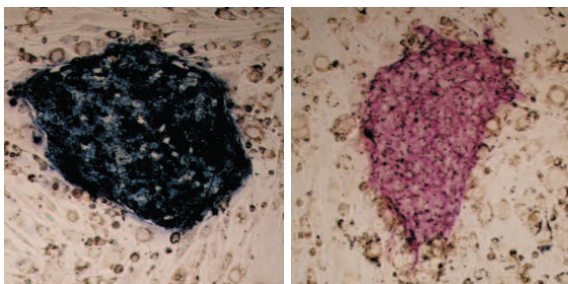
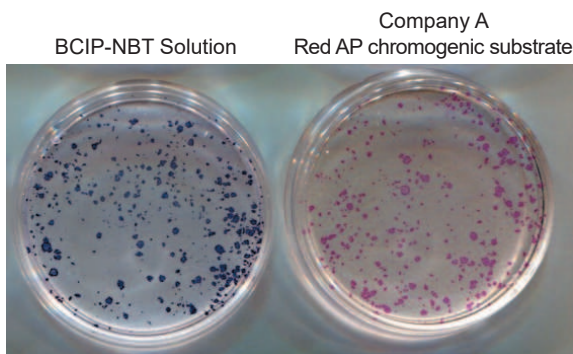
BCIP-NBT Solution (Ready To Use)

A ready to use solution of chromogenic substrate of alkaline phosphatase (AP). It can be used in a wide range of applications, such as detection of alkaline phosphatase (AP) bound by antigen-antibody reaction and endogenous alkaline phosphatase (AP) of stem cells and osteoblasts, in blotting and tissue or cell staining.

- Ready to use
- Can be used in wide range of applications such as immunochemical staining or endogenous AP staining.
- Can be used in in situ hybridization (ISH), as it is tested for DNase and RNase.

Application 1: Endogenous AP staining of iPS cells and osteoblasts

● iPS cell



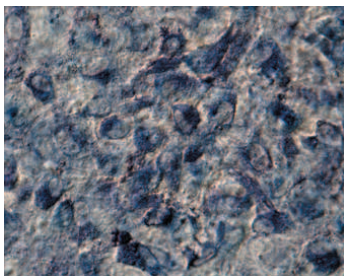
<Experiment conditions>

Cell : iPS cell (01B7 line*), feeder cell (SNL)
 Fixation : 4%-Paraformaldehyde Phosphate Buffer Solution (#09154)
 10 minutes at room temperature
 Staining : 60 minutes at room temperature
 Detection : (Upper) Scanner (Seiko Epson)
 (Lower) Optical microscope (Olympus #BX50), 10x magnification

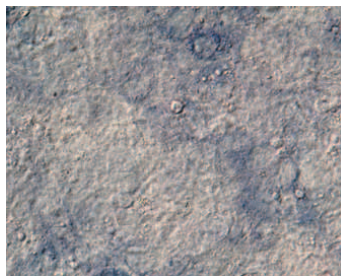
*Takahashi, K. et al. Cell. 2007, 131(5), p. 861-872.

● Osteoblasts

Added BMP-2 (100 ng/mL)
 Cultured for 3 weeks



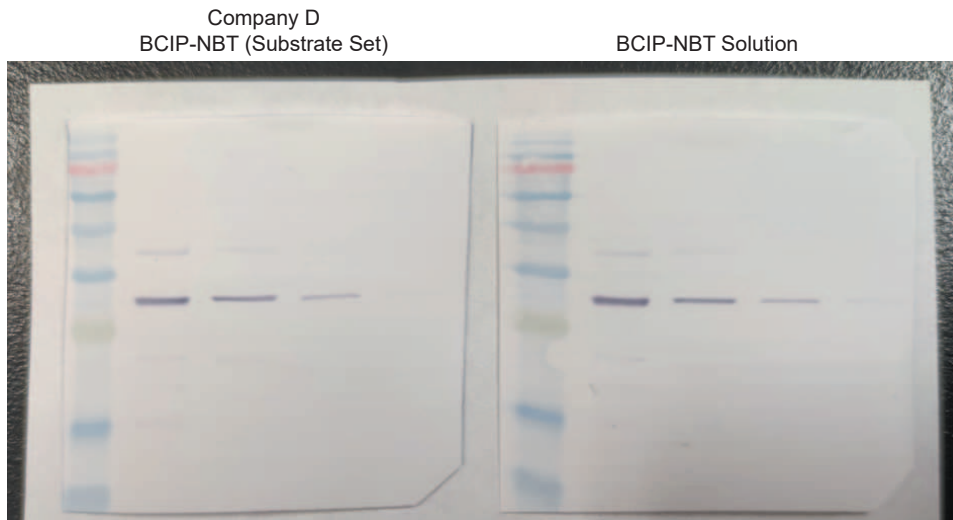
No BMP-2
 Cultured for 3 weeks



<Experiment conditions>

Cell : MC3T3-E1 Cell
 Fixation : 4%-Paraformaldehyde Phosphate Buffer Solution (#09154) 10 minutes in room temperature
 Staining : BCIP-NBT Solution (Ready To Use), 30 minutes at room temperature
 Detection : Optical microscope (Olympus # BX50) by differential interference, 50x magnification

Application 2: Western blotting detection of UCP1 protein from mouse brown adipose tissue



<Experiment conditions>

Sample : Protein from mouse brown adipose tissue (From left 30 μ g, 10 μ g, 3 μ g, 1 μ g)
 Blotting : 5% skim milk/TBS at room temperature for 1 hour.
 Primary antibody : Anti-UCP1 antibody (Rabbit) (Self-preparation) 2,000x diluted
 Diluted using 5% skim milk/TBS, refrigerated overnight.
 Secondary antibody : Anti-Rabbit IgG (Fc), AP Conjugate(Promega #S373B) 5,000x diluted
 Diluted using 5% skim milk/TBS, left at room temperature for 1 hour.
 Detection : 1 minute for each staining solution.

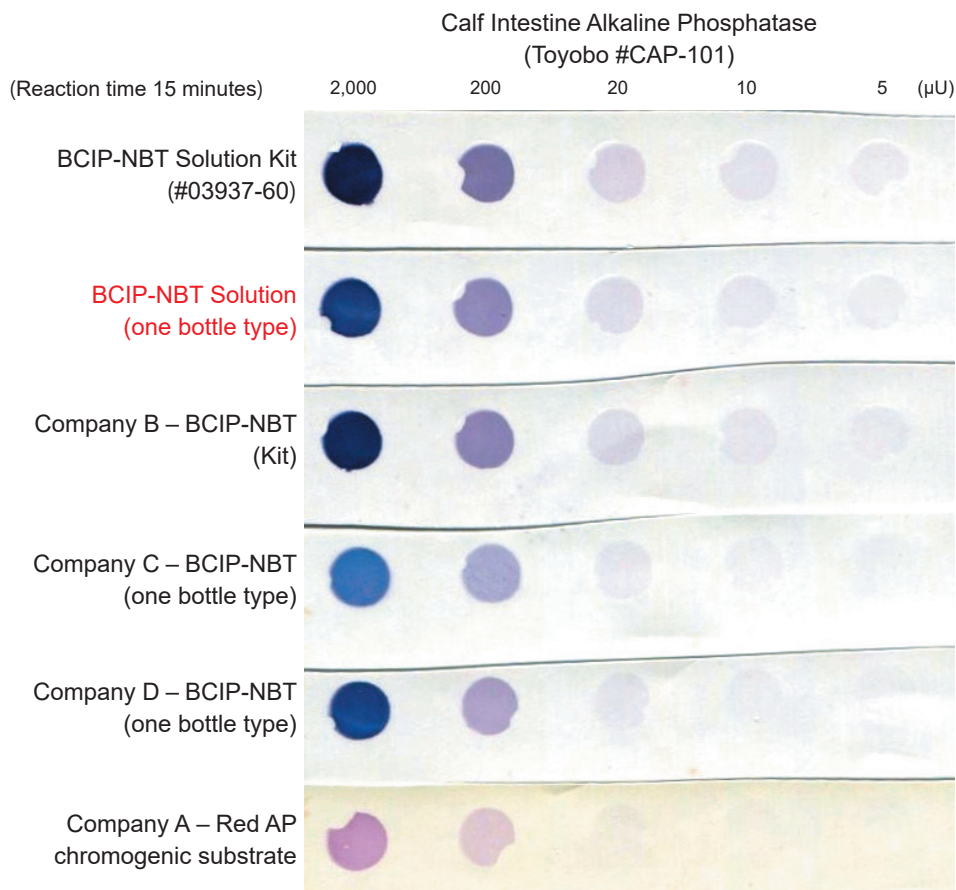
【User evaluation】

BCIP-NBT (Substrate Set) from company D is 2 bottles type which takes time to making our own buffer. On the order hand, 1 bottle type of BCIP-NBT Solution (Ready To Use) is convenience and results as good as others.

Data reference: Professor Goto, Tsuyoshi, Department of Food and Biological Sciences, Graduate School of Agriculture, Kyoto University.

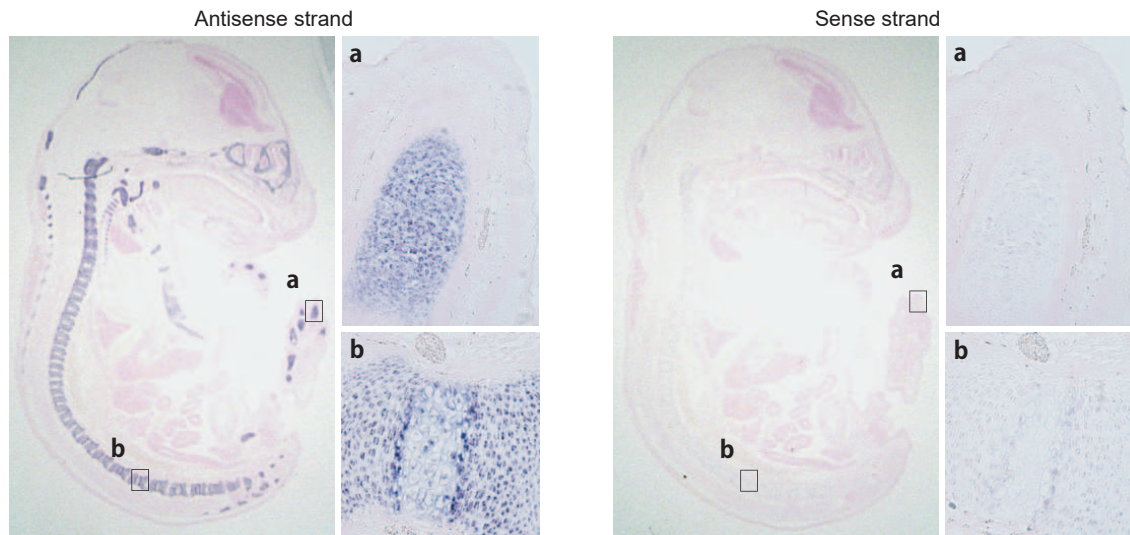
Comparison: Detection sensitivity by dot blotting

Compare with other brands, BCIP-NBT Solution has equal or better sensitivity.



Application 3: In situ hybridization (ISH)

Detecting IX type collagen ($\alpha 1$) mRNA from fetal mouse paraffin embedded section. The product can be used for high sensitivity detection, such as is required with in situ hybridization (ISH).



<Experiment conditions>

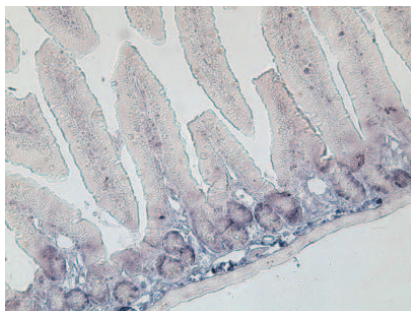
Tissue : Paraffin section from fetal mouse (16.5 days) for ISH (Genostaff #SMPS-08-5)
 Hybridization : RNA probe for DIG tag mouse Col9a1 ISH (Genostaff #MP-C-023)
 Blocking : Blocking One Histo (#06349-64) at room temperature for 10 minutes
 Primary antibody : Anti-Digoxigenin-AP, Fab fragments from sheep (Roche #11093274910) 1:2,000
 Diluent: tTBS / Blocking One Histo in 19:1 ratio
 Staining : BCIP-NBT Solution (Ready To Use), 4 hours at room temperature; Counterstain: Kernechtrot dye, 10 seconds at room temperature
 Observation : Enclosed by CC/Mount (Diagnostic BioSystems #K002) and Entellan® new (Merck #107961), observed by Zoom Stereomicroscope MF2.6x (AXEL #1-1925-02), microscope MF 50x (Olympus #BX50)

Application 4: Immunohistochemical staining

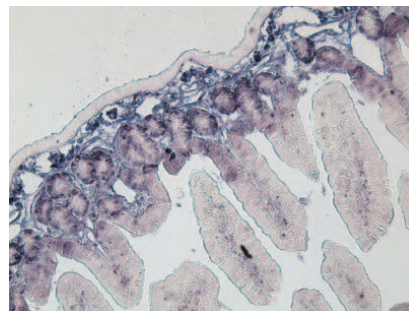
Detecting PCNA from mouse small intestine paraffin embedded section. If there is insufficient sensitivity, extending reaction time can result in good sensitivity.

(Reaction time)

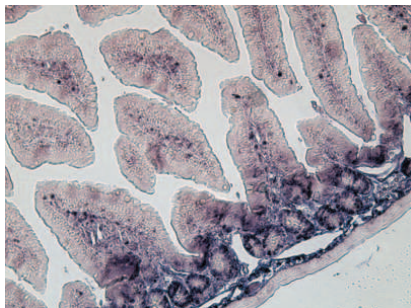
5 minutes



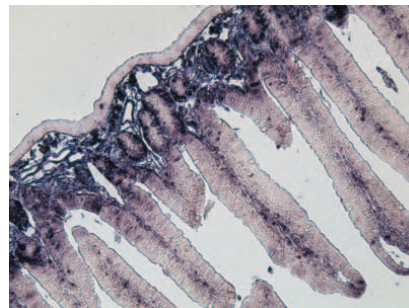
10 minutes



15 minutes



30 minutes

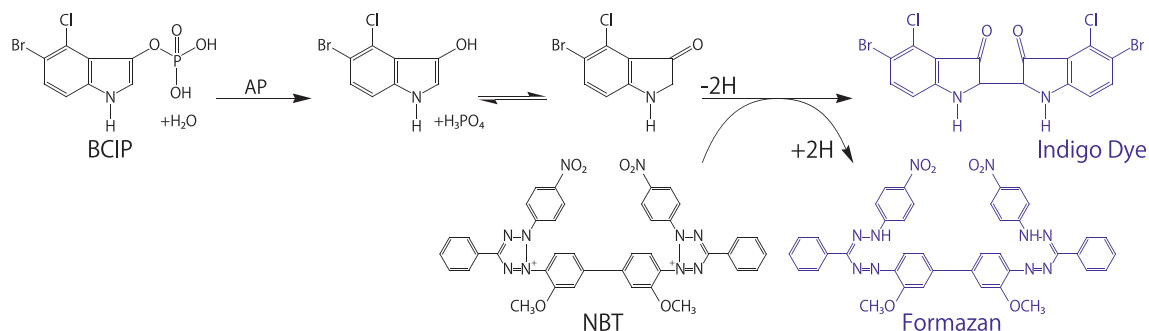


<Experiment conditions>

Tissue : Small intestine paraffin section from mouse
 Antigen activation : HistoVT One (10x, pH 7.0) (#06380) at 90°C for 20 minutes
 Blocking : Blocking One Histo (#06349-64) at room temperature for 10 minutes
 Primary antibody : PCNA Antibody (Novus #NB100-456) 1:500
 Diluent: tTBS / Blocking One Histo in 19:1 ratio
 Secondary Antibody : Goat anti-Rabbit IgG (H+L) Secondary Antibody [Alkaline Phosphatase] (Pre-adsorbed) (Novus #NB7181) 1:1,000
 Used tTBS as an antibody diluent
 Staining : BCIP-NBT Solution (Ready To Use), reacted at room temperature for time shown above
 Observation : Enclosed by CC/Mount (Diagnostic BioSystems #K002), observed by microscope MF 25x (Olympus #BX50)

Measurement principle

BCIP, a chromogenic substrate for alkaline phosphatase (AP), produces an indigo color by reaction. In the same reaction, NBT is reduced and turns into an insoluble purple precipitate.



Usage

Procedure for staining endogenous alkaline phosphatase (AP). For other applications of usage, please refer product instruction.

1. Fix cells with a fixing solution such as paraformaldehyde
2. Wash fixing solution with water.
3. Pour BCIP-NBT Solution slowly, and leave at room temperature to react.
(For 35mm dish, please use 1mL of BCIP-NBT Solution)
4. When an appropriate stained image is obtained, wash with water to stop the reaction.
5. Observe it enclosed or after air drying.

Ordering information

| Product Name | Grade | Storage | Catalog Number | PKG Size |
|----------------------------------|-------|--------------|----------------|----------|
| BCIP-NBT Solution (Ready To Use) | SP | Refrigerator | 19880-84 | 100 mL |

Related product

| Product Name | Grade | Storage | Catalog Number | PKG Size |
|---|-------|------------------|----------------|-----------|
| Blocking One Histo | SP | Refrigerator | 06349-64 | 50 mL |
| HistoVT One (10x, pH 7.0) | SP | Room temperature | 06380-76 | 100 mL |
| | | | 06380-05 | 500 mL |
| 4%-Paraformaldehyde Phosphate Buffer Solution | SP | Refrigerator | 09154-14 | 5 X 10 mL |
| | | | 09154-56 | 100 mL |
| | | | 09154-85 | 500 mL |

For research use only, not intended for diagnostic or drug use.