Protocol

Detecting Alkaline Phosphatase–Labeled Cells

Scott J. Rodig

Different substrates are available for detection of cells labeled with alkaline phosphatase. Naphthol-AS-BI-phosphate (NABP)/New Fuchsin produces an intense red product that is insoluble in alcohols as well as aqueous solutions and is compatible with a range of histochemical stains. Bromochloroindolyl phosphate/nitro blue tetrazolium (BCIP/NBT) is the most commonly used of the chromogenic substrates for alkaline phosphatase. It is somewhat more sensitive than NABP/New Fuchsin but gives less contrast during microscopic observation.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Cells labeled with alkaline phosphatase–labeled antibodies

Use eukaryotic alkaline phosphatase, which is readily inactivated by EDTA. The bacterial enzyme is difficult to stop, causing overdevelopment and leading to high background.

Reagents for detection using BCIP-NBT (Steps 11–16)

Alkaline phosphatase buffer for staining <R>

Bromochloroindolyl phosphate (disodium salt) (BCIP; 0.5 g in 10 mL of 100% dimethylformamide)

BCIP stock is stable for at least 1 yr at 4°C.

Nitro blue tetrazolium (0.5 g in 10 mL of 70% dimethylformamide)

NBT stock is stable for at least 1 yr at 4°C.

PBS containing 20 mM EDTA

Reagents for detection using NABP-NF (Steps 1–10)

Dimethylformamide

EDTA (20 mM)

HCl (2 N)

Naphthol AS-TR phosphate (sodium salt)

New Fuchsin

Sodium nitrate

Tris (0.2 M, pH 9.0)

From the Antibodies collection, edited by Edward A. Greenfield.

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Equipment

Dish or tray to hold cells for staining (for detection with BCIP-NBT)
Shaker (for detection with BCIP-NBT)

METHOD

Detecting Alkaline Phosphatase–Labeled Cells Using NAPB-NF

1. Dissolve 1 mg of New Fuchsin in 0.25 mL of 2 N HCl.
2. Dissolve 1 mg of sodium nitrate in 0.25 mL of H₂O.
3. Dissolve 10 mg of naphthol AS-TR phosphate in 0.2 mL of dimethylformamide.
4. Mix the New Fuchsin solution with the sodium nitrate solution. Shake for 1 min.
5. Add the mixture to 40 mL of 0.2 M Tris (pH 9.0).
6. Add the naphthol AS-TR solution to the above mixture.
7. Apply to specimen and incubate for 10–40 min at room temperature.
8. Stop the reaction by washing with 20 mM EDTA.
9. (Optional) Counterstain if necessary (see Protocol: **Counterstaining, Mounting, and Photographing Stained Cells** [Rodig 2019]).
10. Mount in DPX (see Protocol: **Counterstaining, Mounting, and Photographing Stained Cells** [Rodig 2019]).

Detecting Alkaline Phosphatase–Labeled Cells Using BCIP-NBT

11. Just before developing, prepare fresh substrate solution. Add 66 µL of NBT stock to 10 mL of alkaline phosphatase buffer. Mix well and add 33 µL of BCIP stock. Use within 1 h.
12. Place the washed cells in a suitable container. Add enough substrate solution to cover the cells (for a 100-mm dish, 3–5 mL is appropriate).
13. Develop at room temperature with agitation until the stain is suitably dark. Periodic monitoring under the microscope may be necessary for some antigens. A typical incubation would be ~30 min.
14. To stop the reaction, rinse with PBS containing 20 mM EDTA, chelating the Mg²⁺ ions.
15. (Optional) Counterstain if necessary (see Protocol: **Counterstaining, Mounting, and Photographing Stained Cells** [Rodig 2019]).
16. Wash in H₂O and mount in DPX (see Protocol: **Counterstaining, Mounting, and Photographing Stained Cells** [Rodig 2019]).

RECIPE

Alkaline Phosphatase Buffer

NaCl (100 mM)
MgCl₂ (5 mM)
Tris-Cl (100 mM, pH 9.5)

Can be made up from stocks and autoclaved. Buffer is stable at room temperature.

REFERENCES

Rodig SJ. 2019. Counterstaining, mounting, and photographing stained cells. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot099770.

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