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Alkaline Phosphatase Staining Kit

01/20

(Catalog #K2035-50; 50 assays; Store at -20°C)

I. Introduction:

The Alkaline Phosphatase (AP) Staining Kit provides a quick and easy method to stain Alkaline Phosphatase in pluripotent stem cells. It is based on the ability of Alkaline Phosphatase to hydrolyze the phosphate group on the substrate, 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) to form a blue colored intermediate. Nitro Blue Tetrazolium (NBT) then oxidizes the intermediate to form an insoluble, dark blue dimer. Because stem cells/pluripotent stem cells have increased AP activity, the AP Staining Kit can be used to identify and screen Alkaline Phosphatase activity in these cells. The staining method is simple, rapid, cost-effective and reliable. The entire procedure can be performed in the same microtiter plate and does not require any extra steps such as fixing or harvesting the cells. The assay can be also used as the initial step to test the pluripotency of cells and to monitor osteogenic differentiation of pluripotent/stem cells.

II. Applications:

- Detection of alkaline phosphate activity in stem cells/pluripotent stem cells, HeLa, HepG2, HEK293 cells, osteoblasts and osteocytes.
- Screening for pluripotent cells induced by various factors.
- AP Staining in stem cells/pluripotent stem cells or stem cells undergoing osteogenesis.

III. Sample Types:

- Stem cells
- Induced pluripotent stem cells
- Cell lines containing Alkaline Phosphatase activity

IV. Kit Contents:

Components	K2035-50	Cap Color	Part Number
Wash Buffer	50 ml	NM	K2035-50-1
AP Staining Reagent	5 bottles	Blue	K2035-50-2

V. User Supplied Reagents and Equipment:

- Sterile, tissue culture treated 48-well clear plate with clear bottom and lid
- Multichannel or single channel Pipettor
- Light Microscope for live imaging

VI. Reagents Preparation and Storage:

Store the kit at -20°C, protected from light.

- **Wash Buffer:** Warm to room temperature (RT) before use. Store at -4°C.
- **AP Staining Reagent:** Before use, add 1.1 ml dH_2O to one bottle. Mix well to make 1 X AP Staining Reagent solution. Discard the remaining AP Staining solution after use.

VII. Alkaline Phosphatase Staining Protocol:

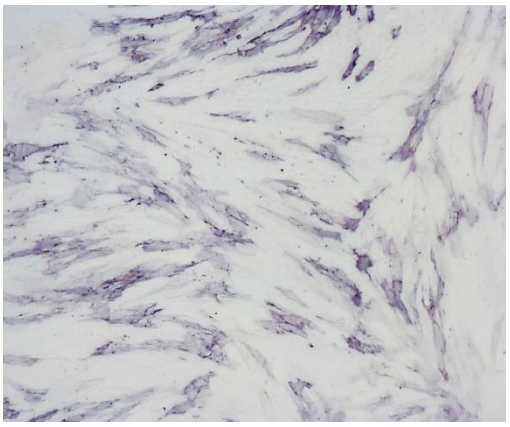
This assay was developed for a 48 well plate but can be used for other plates by adjusting the volume of the stain. For 96-well plates, use 50 µl per well. For 24-well plates, use 250 µl per well. For 12-well plates, use 500 µl per well. For 6-well plates, use 1.0 ml per well

1. Culture cells as desired or as instructed by the differentiation/maintenance protocol.
2. Prepare the AP Staining Reagent as described above.
3. Carefully remove the media from the cultured cell wells.
4. Gently add 300 µl Wash Buffer, tilt the plate and carefully remove all of the Wash Buffer using a pipette. **Do not use vacuum.**
5. Carefully add 100 µl of AP Staining Reagent solution completely and evenly to cover the cells in each well. Incubate for 10-30 min at 37°C.
6. Wash gently with 300 µl Wash Buffer per well, tilt and remove the Wash Buffer with a pipette. Wash 2 times.
7. Add 100 µl of Wash Buffer and image using Light Microscope.

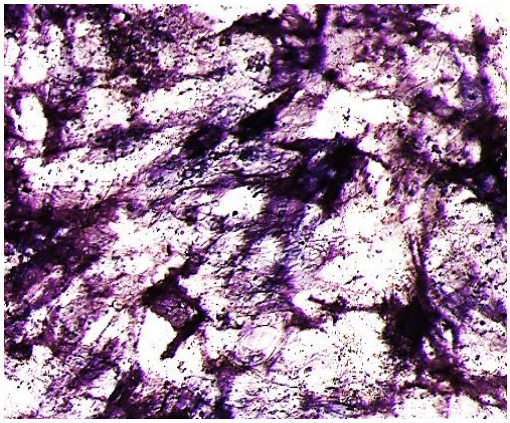
Notes:

- This assay was developed in Human bone marrow-derived mesenchymal stem cells and human mesenchymal stem cells undergoing osteogenesis, (cell density ranges from 5000 to 10,000 cells per well).
- Do not pipette directly into the cells. Pipette to the side of the wells and mix by rotating.
- Different cell types may require an adjustment in the cell numbers to maintain the optimal cell density range.
- Phenol Red, other culture media components and serum do not interfere with the AP staining protocol.

A)



B)



Figures: A). Alkaline Phosphatase staining in Human bone marrow-derived mesenchymal stem cell culture. **B).** Staining of human bone-marrow-derived mesenchymal stem cells undergoing osteogenesis. Dark bluish-purple precipitation represents Alkaline Phosphatase staining. AP staining was performed according to the kit protocol.

VIII. Related Products:

Alkaline Phosphatase Assay Kit (K422)
LDH-Cytotoxicity Assay Kit (K311)
Bioluminescence Cytotoxicity Assay Kit (K312)
Live/Dead Cell Staining Kit (K355)
Cell Proliferation Assay (K307)
Lipid (Oil Red O) Staining Kit (K580)
Phosphate Assay Kit (Fluorometric) (K420)
Acid Phosphatase Assay Kit (K411)
Phosphate Assay Kit (Colorimetric) (K410)
Senescence Detection Kit (K911)
Annexin V-FITC Apoptosis Detection Kit (K101)

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