ab284936 – Alkaline Phosphatase Staining Kit

For the detection of alkaline phosphate activity in stem cells/pluripotent stem cells, HeLa, HepG2, HEK293 cells, osteoblasts and osteocytes.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284936

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.

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.

Sample Types

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

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Wash Buffer II	50 mL	-20°C
AP Staining Reagent	5 vials	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

Store the kit at -20°C. Protect from light. Read the entire protocol before performing the assay.

Wash Buffer II: Warm to room temperature (RT) before use. Store at -4°C.

AP Staining Reagent: Before use, add 1.1 mL dH2O to one bottle. Mix well to make 1 X AP Staining Reagent solution. Discard the remaining AP Staining solution after use.

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Assay Protocol

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 II, tilt the plate and carefully remove all of the Wash Buffer II 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 II per well, tilt and remove the Wash Buffer II with a pipette. Wash 2 times.
7. Add 100 µL of Wash Buffer II and image using Light Microscope.

Δ Notes:

a) *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).*
b) *Do not pipette directly into the cells. Pipette to the side of the wells and mix by rotating.*
c) *Different cell types may require an adjustment in the cell numbers to maintain the optimal cell density range.*
d) *Phenol Red, other culture media components and serum do not interfere with the AP staining protocol.*

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)