COS-0105

Alkaline Phosphatase Staining Kit

(To stain 96-, 48-, 24-, 12-, 6-well plates, Store at -20°C)

Introduction:

Alkaline phosphatase (ALP) activity is high in liver, bone and kidney. This makes it a useful tool for monitoring processes in which it is involved. Stem cells express ALP with high activity when in the pluripotent or undifferentiated state. As differentiation proceeds, ALP activity declines. The use of artificial substrates such as BCIP/NBT results in a dark blue precipitate allowing visualization of ALP activity in cultured cells, providing a reliable indicator of undifferentiated stem cells within a population. AkrivisBio's Alkaline Phosphatase Staining Kit utilizes the BCIP/NBT protocol originally developed by McGadey in 1970 (ref 1) based on earlier indoxyl substrates. In the stain, BCIP is hydrolyzed to an indoxyl which reduces the NBT to a darkly colored formazan. The staining protocol is a simple procedure which can provide results in under an hour.

Staining Kit Components:

Wash Buffer	50 ml	NM	COS-0105A
ALP Staining Reagent	5 bottles	Blue	COS-0105B

User Supplied Materials:

Sterile, tissue culture treated 48-well clear bottom plate and lid.
Multichannel Pipettor

Storage and Handling:

This assay is designed for wells of a 48 well plate but can be used for other plates by adjusting the volume used appropriately:

	Wash Buffer	ALP Stain
96-well plate	50 µl per well	150 µl per well
48-well plate	100 µl per well	300 µl per well
24-well plate	250 µl per well	600 µl per well
12-well plate	500 µl per well	1.0 ml per well
6-well plate	1.0 ml per well	2.0 ml per well

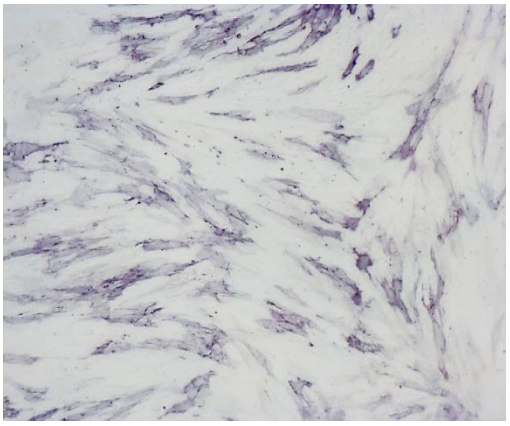
Store the unopened kit at -20°C.
Wash Buffer: Warm to room temperature before use. Store at 4°C.
ALP Staining Reagent: Reconstitute one bottle with 1.1 ml DI H_2O. Mix well to make ALP Stain working solution. Prepare only the amount needed for immediate requirements as the reconstituted stain cannot be stored. Discard any remaining ALP Stain working solution after use.

Staining Protocol:

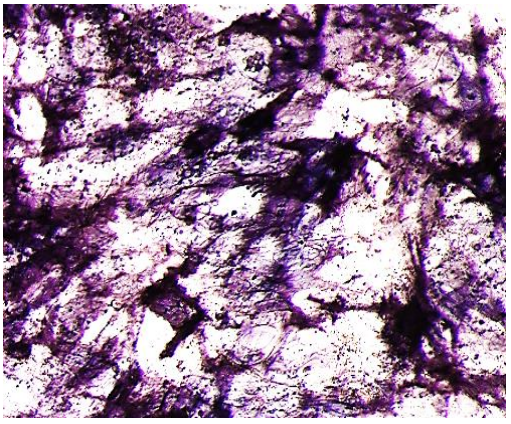
1. Culture cells as necessary.
2. Remove the media from the culture plate wells, gently.
3. Add 300 µl Wash Buffer, tilt the plate and remove all of the Wash Buffer gently, using a pipette. **Do not use a vacuum.**
4. Add 100 µl of ALP Stain working solution to all wells using a multichannel pipettor. Work quickly but carefully.
5. Tap the plate to cover the cells evenly in all wells.
6. Incubate for 10-30 min at 37°C.
7. Carefully remove the ALP stain working solution
8. Wash gently 2X with 300 µl Wash Buffer per well. Tilt and remove the Wash Buffer with a pipette.
9. Add 100 µl of Wash Buffer and image using a Light Microscope at ~ 200X.

Notes:

Do not pipette directly into the cells. Pipette to the side of the wells and mix by rotating.
Phenol Red, other culture media components and serum do not interfere with the AP staining protocol.



LP staining in mesenchymal stem cells.



ALP Stain of mesenchymal stem cells undergoing osteogenesis.

Reference:
1) McGadey, J., Histochemie,;**23**(2):180-4. 1970 (doi: 10.1007/BF00305851)

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