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ab242286
Alkaline Phosphatase Staining Kit (Red)

View Alkaline Phosphatase Staining Kit datasheet:
www.abcam.com/ab242286

For the for monitoring of ES cell undifferentiation/ differentiation.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Like mouse Embryonic stem (ES) cells, human ES cells express high levels of membrane alkaline phosphatase (AP) and Oct-4, a transcriptional factor critical to inner cell mass (ICM) and germline formation. However, unlike mouse ES cells, hES cells do not express stage-specific embryonic antigen (SSEA-1). In addition, prolonged propagation of hES cells is typically achieved by co-culture with primary mouse embryonic fibroblasts (MEFs) serving as feeder cells. Human ES cell lines are not able to maintain their undifferentiated state in the absence of supporting feeder layer cells, even when exogenous cytokines such as leukemia inhibitory factor (LIF) and gelatin-coated plates are used.

Marker Name	Mouse ES Cells	Mouse EG Cells	Human ES Cells	Human EG Cells	Human EC Cells
AP	Yes	Yes	Yes	Yes	Yes
SSEA-1	Yes	Yes	No	Yes	No
SSEA-4	No	No	Yes	Yes	Yes
TRA-1-60	No	No	Yes	Yes	Yes
TRA-1-81	No	No	Yes	Yes	Yes
Oct-4	yes	yes	yes	unknown	Yes

ES Cell = Embryonic stem cell

EG Cell = Embryonic germ cell

EC Cell = Embryonic carcinoma cell

Figure 1: Comparison of mouse and human pluripotent stem cells.

Although stem cells from different origins require different growth conditions for self-renewal, and display different cell surface markers, AP is the most widely used stem cell marker.

Alkaline Phosphatase Staining Kit (Red) (ab242286) provides an efficient system for monitoring ES cell undifferentiation/ differentiation through AP activity by immunocytochemistry staining.

2. Protocol Summary

Culture mouse ES cells in medium containing LIF; human ES cells on a MEF feeder layer.

Aspirate medium and wash with PBST.

Aspirate PBST and add Fixing Solution, incubate at room temperature for 2 mins.

Remove Fixing Solution and wash with PBST.

Aspirate wash and add freshly prepared AP Staining Solution, incubate at room temperature for 15-30 mins, protected from light.

Remove AP Staining Solution and wash with 1X PBS.

Either overlay cells with 1X PBS/ 20% glycerol at store at +4°C or count the red stained colonies vs. colorless colonies using a light microscope.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at 4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Fixing Solution	50 mL	4°C
AP Staining Solution A	20 mL	4°C
AP Staining Solution B	20 mL	4°C

5. Materials Required, Not Supplied

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

- Human or mouse embryonic stem cells and culture medium
- 1X PBS
- 1X PBST (1X PBS containing 0.05% Tween-20)
- Deionized water
- Light microscope

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 AP Staining Solution

6.1.1 Prepare a 1X working solution freshly before the assay.

6.1.2 Mix equal volume of AP Staining Solution A and AP Staining Solution B. The volume of AP Staining Solution needed is based on the number of samples. The chart below is suggested for samples in a 24-well plate, and may be modified accordingly to suit other culture plate sizes:

Reagents	Half plate (12 samples)	1 plate (24 samples)	4 plates (96 samples)
Staining Solution A	2.4 mL	4.8 mL	9.6 mL
Staining Solution B	2..4 mL	4.8 mL	9.6 mL
Total	4.8 mL	9.6 mL	19.2 mL

7. Assay Procedure

7.1 24-Well Plate:

7.1.1 Culture mouse ES cells in medium containing LIF; alternatively, culture human ES cells on a MEF feeder layer.

7.1.2 Gently aspirate the medium from the ES cells and wash the cells with 1 mL of 1X PBST. Aspirate the wash solution.

7.1.3 Add Fixing Solution to the cells, 0.4 mL per well for a 24-well plate. Incubate at room temperature for 2 mins.

7.1.4 Remove the fixing solution and wash the fixed cells twice with 1 mL of 1X PBST.

7.1.5 Aspirate the final wash and add 0.4 mL per well of freshly prepared AP Staining Solution.

7.1.6 Incubate the cells at room temperature for 15-30 mins, protected from light.

7.1.7 Remove the AP Staining Solution, and then wash the stained cells twice with 1 mL of 1X PBS. Store cells in 1X PBS at 4°C. For long-term storage, overlay the cells with 1X PBS containing 20% Glycerol. Store at 4°C.

7.1.8 Count the red stained cell colonies (undifferentiated ES cells) vs. colorless colonies (differentiated ES cells) using a light microscope.

8. Typical Data

The following figure demonstrates typical results with AP Staining Solution. One should use the data below for reference only. This data should not be used to interpret actual results.

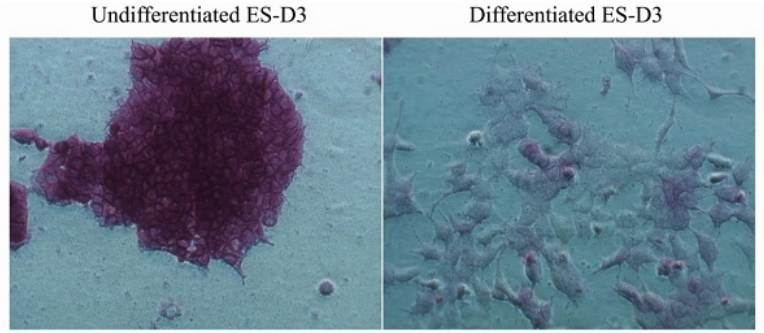

Figure 1. AP staining of ES Cells. Murine embryonic stem cells (ES-D3) are maintained in an undifferentiated stage on gelatin-coated dishes in the presence of LIF, as indicated by the high AP activity. To induce differentiation, LIF was withdrawn over a period of several days; various differentiation events were observed (cells became flattened and enlarged with reduced proliferation). At the end of day 5, AP staining of undifferentiated cells was performed as described in the Assay Protocol.

9. Notes

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