Product Manual

StemTAG™ Alkaline Phosphatase Activity Assay Kit (Colorimetric)

Catalog Number

CBA-301 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
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Introduction

Embryonic stem (ES) cells are continuous proliferating stem cell lines of embryonic origin first isolated from the inner cell mass (ICM). Two distinguishing features of ES cells are their ability to be maintained indefinitely in an undifferentiated state and their potential to develop into any cell within the body. Based on previous methods developed for mouse ES cells, human ES cell lines were first established by Dr. James Thomson and colleagues. Like mouse ES cells, human ES cells express high levels of membrane alkaline phosphatase (AP) and Oct-4, a transcriptional factor critical to 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 coculture 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	√	√	√	√	√
SSEA-1	√	√	–	√	–
SSEA-4	–	–	√	√	√
TRA-1-60	–	–	√	√	√
TRA-1-81	–	–	√	√	√
Oct-4	√	√	√	unknown	√
ES Cell = Embryonic stem cell EG Cell = Embryonic germ cell EC Cell = Embryonic carcinoma cell					

Table 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 (see Table 1), AP is the most widely used stem cell marker. The StemTAG™ Alkaline Phosphatase Activity Assay Kit provides an efficient system for monitoring ES cell undifferentiation/ differentiation through AP activity by quantitative assay.

Related Products

1. CBA-300: StemTAG™ Alkaline Phosphatase Staining Kit
2. CBA-312: MEF Feeder Cells (Puromycin-resistant)
3. CBA-316: SNL Feeder Cells
4. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

Kit Components

1. StemTAG™ AP Activity Assay Substrate (Part No. 30004): One bottle – 5 mL
2. Cell Lysis Buffer (Part No. 30005): One bottle – 20 mL
3. 10X Stop Solution (Part No. 30006): One bottle – 10 mL
4. AP Activity Assay Standard (Part No. 30007): One tube – 1 mL of 5 mM p-Nitrophenol

Materials Not Supplied

1. Human or Mouse Embryonic Stem Cells and Culture Medium
2. 1X PBS
3. 1X PBST (1X PBS containing 0.05% Tween-20)

Storage

Store all components at 4ºC.

Preparation of Reagents

- 1X Stop Solution: Prepare a 1X Stop Solution by diluting the provided 10X stock 1:10 in deionized water. Store the diluted solution at room temperature.

Preparation of Standard Curve

1. Prepare a 10-fold dilution of the AP Activity Assay Standard (5 mM pNP) with 1X Stop Solution. For example, in a microtube, add 100 µL of the AP Activity Assay Standard to 900 µL of 1X Stop Solution, mixing well.
2. Prepare 2-fold serial dilutions of the AP Activity Assay Standard solution with 1X Stop Solution. For example, label ten microtubes #1 to #10, add 0.5 mL of 1X Stop Solution to each tube. Transfer 0.5 mL of the 10-fold diluted AP Assay Standard Solution (0.5 mM final) to tube #1, mix well and transfer 0.5 mL of the mixture to tube #2. Repeat until tube #9, and use tube #10 as blank.
3. Transfer 150 µL of each dilution, in duplicate, to a 96-well plate, read the absorbance of each well at 405 nm.

Assay Protocol

1. Culture mouse ES cells in medium containing LIF; alternatively, culture human ES cells on a MEF feeder layer.
2. Gently aspirate the medium from the ES cells and wash the cells twice with cold PBS. Aspirate the wash solutions.
3. Lyse the cells in Cell Lysis Buffer (0.5 mL for a 35 mm dish).
4. Incubate for 10 minutes at 4ºC, remove the solution and spin down the cell debris at 12,000 X g for 10 minutes. Save the supernatant as cell lysate. Perform a BCA assay or other protein assay to determine the protein concentration of the cell lysate.
5. Add 50 µL of cell lysate to a 96-well plate. In addition, prepare blank wells that contain 50 µL Cell Lysis Buffer. We recommend testing samples in triplicate.
6. Initiate the reaction by adding 50 µL of StemTAG™ AP Activity Assay Substrate. Incubate for 10-30 minutes at 37ºC.
7. Stop the reaction by adding 50 µL of 1X Stop Solution and mix by placing the plate on an orbital plate shaker for 30 seconds.
8. Read the absorbance of each well at 405 nm.

Example of Results

The following figures demonstrate typical results with the StemTAG™ Alkaline Phosphatase Activity Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

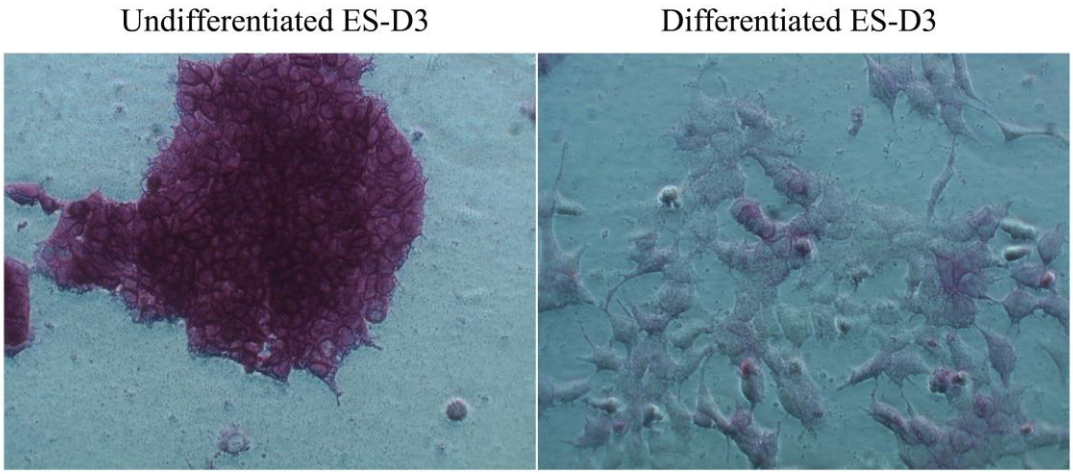

Figure 1: AP staining of ES Cells. Murine embryonic stem cells (ES-D3) are maintained in an undifferentiated stage on gelatin-coated dishes in the presence of LIF, as indicated by the high AP activity. To induce differentiation, LIF was withdrawn over a period of several days; various differentiation events were observed (cells became flattened and enlarged with reduced proliferation). At the end of day 5, AP staining of undifferentiated cells was performed with the StemTAG™ Alkaline Phosphatase Staining Kit (Cat # CBA-300).

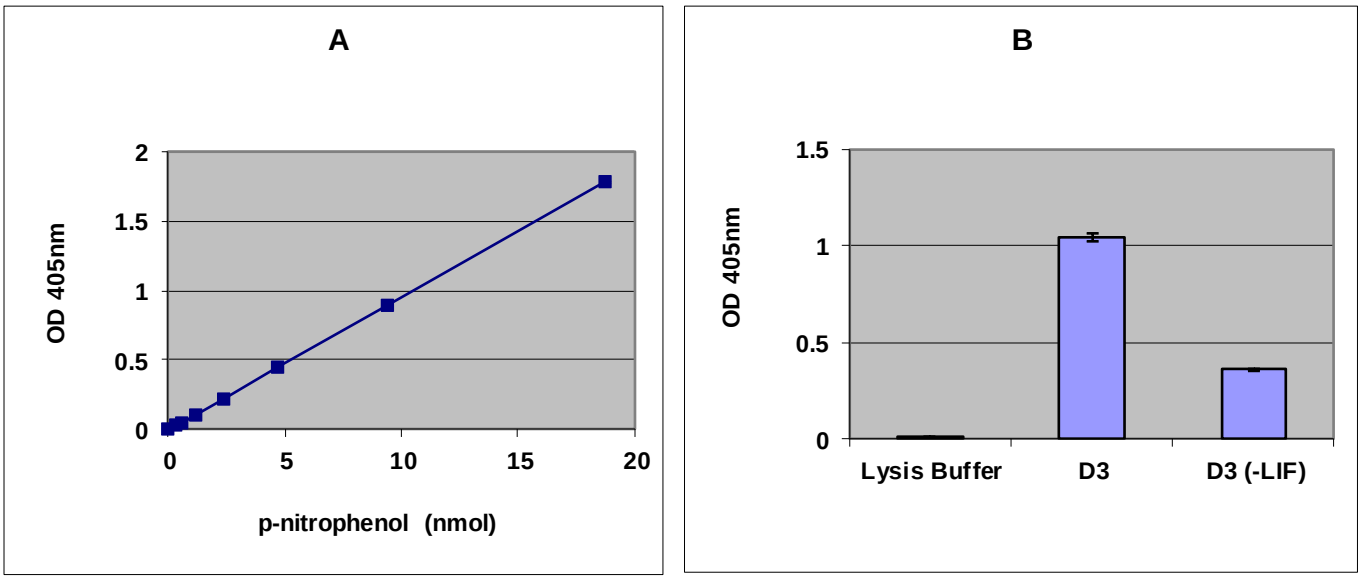

Figure 2: **pNP Standard Curve and AP Activity Assay. A**: A serial 2-fold dilution of pNP standard was prepared in 1X Stop Solution, and the absorbance of each dilution was measured at 405 nm. **B**: Mouse embryonic D3 cells were grown in the presence or absence of LIF for 5 days. 10 µg of cell lysate was assayed for AP activity according to the Activity Assay Instructions.

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Recent Product Citations

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