Product Manual

StemTAG™ Alkaline Phosphatase Complete Kit

Catalog Number

CBA-302 **2 x 100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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.

Table 1. Comparison of Mouse and Human Pluripotent Stem Cells

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

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 Complete Kit provides an efficient system for monitoring ES cell undifferentiation/ differentiation through AP activity by both immunocytochemistry staining and quantitative activity assay.

Related Products

1. CBA-300: StemTAG™ Alkaline Phosphatase Staining Kit
2. CBA-301: StemTAG™ Alkaline Phosphatase Activity Assay Kit
3. CBA-325: StemTAG™ Stem Cell Colony Formation Assay
4. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay
5. CBA-304: Total RNA – Murine Embryonic Stem Cell Line D3

6. CBA-305: Total Protein – Murine Embryonic Stem Cell Line D3
7. CBA-312: MEF Feeder Cells (Puromycin-resistant)

Kit Components

1. Fixing Solution (Part No. C30001): One bottle – 50 mL
2. StemTAG™ AP Staining Solution A (Part No. C30002): One amber bottle – 20 mL
3. StemTAG™ AP Staining Solution B (Part No. C30003): One amber bottle – 20 mL
4. StemTAG™ AP Activity Assay Substrate (Part No. C30004): One bottle – 5 mL
5. Cell Lysis Buffer (Part No. C30005): One bottle – 20 mL
6. 10X Stop Solution (Part No. C30006): One bottle – 10 mL
7. AP Activity Assay Standard (Part No. C30007): 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)
4. Deionized Water
5. Light Microscope
6. 96-well Microplate Reader

Storage

Store all components at 4ºC until their expiration dates.

Preparation of Reagents

- StemTAG™ AP Staining Solution: Prepare FRESH 1X StemTAG™ AP Staining Solution by mixing equal volume of StemTAG™ AP Staining Solution A and StemTAG™ AP Staining Solution B. The volume of StemTAG™ 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.

Table 2. Preparation of StemTAG™ AP Staining Solution

Reagents	half plate (12 samples)	1 plate (24 samples)	4 plate (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**

- 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 AP Activity 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: AP Staining (24-Well Plate)

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 with 1 mL of 1X PBST. Aspirate the wash solution.
3. Add Fixing Solution to the cells, 0.4 mL per well for a 24-well plate. Incubate at room temperature for 2 minutes.
4. Remove the fixing solution and wash the fixed cells twice with 1 mL of 1X PBST.
5. Aspirate the final wash, and add 0.4 mL per well of freshly prepared StemTAG™ AP Staining Solution (see Preparation of Reagents section).
6. Incubate the cells at room temperature for 15-30 minutes, protected from light.
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.
8. Count the red stained cell colonies (undifferentiated ES cells) vs. colorless colonies (differentiated ES cells) using a light microscope.

Assay Protocol: AP Activity Assay

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. After a 10-minute incubation 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 37°C.
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.

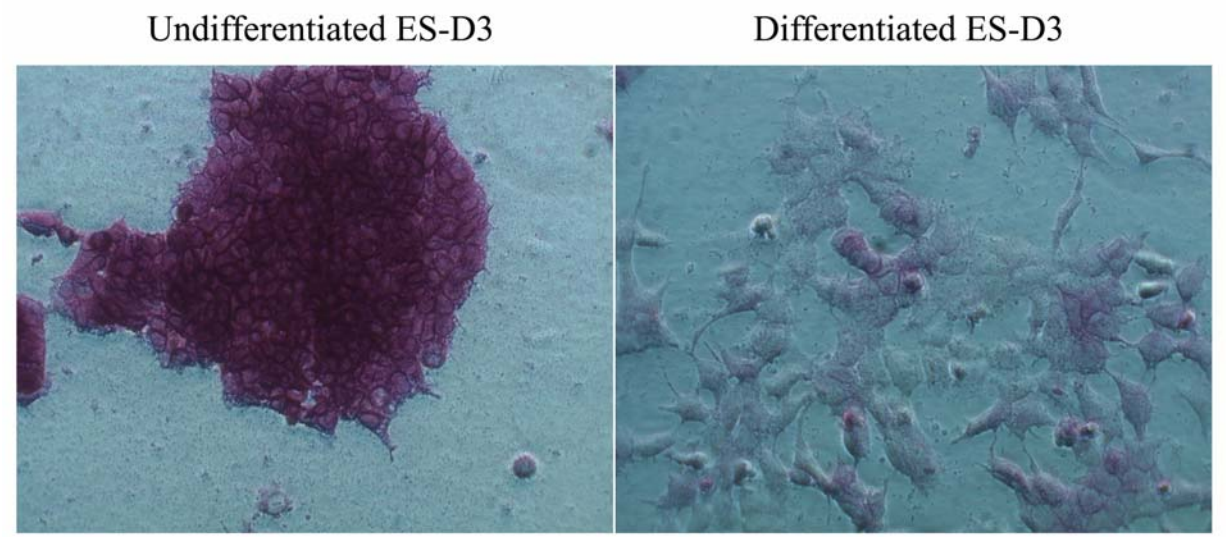

Figure 1: AP staining of ES Cells. Murine embryonic stem cells (ES-D3) are maintained in an undifferentiated stage on gelatin-coated dishes in the presence of LIF, as indicated by the high AP activity. To induce differentiation, LIF was withdrawn over a period of several days; various differentiation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

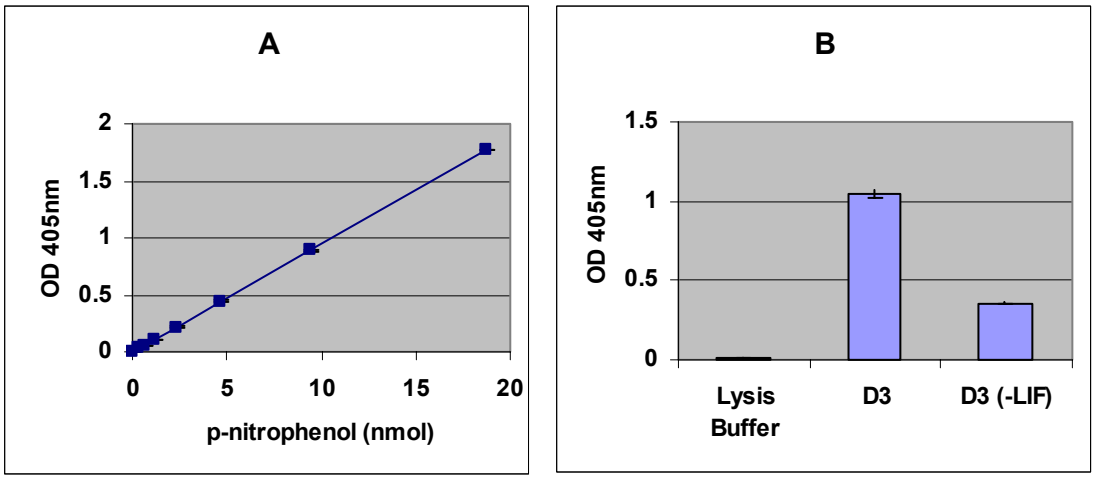

Figure 2: **pNP Standard Curve and AP Activity Assay.** **A**: A serial 2-fold dilution of pNP standard was prepared in 1X Stop Solution, and the absorbance of each dilution was measured at 405 nm. **B**: Mouse embryonic D3 cells were grown in the presence or absence of LIF for 5 days. 10 µg of cell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

References

1. Wobus AM, Holzhausen H, Jäkel P et al. (1984) *Exp Cell Res* 152:212–219.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. (1998) *Science* 282:1145–1147.
3. Smith AG, Nichols J, Robertson M et al. (1992) *Dev Biol* 151:339–351.
4. Reubinoff BE, Pera MF, Fong CY et al. (2000) *Nat Biotechnol* 18:399–404.

Recent Product Citations

1. Dharmacon Application Note: DharmaFect Transfection Reagents for siRNA Transfection into Embryonic Stem Cells (2006). Publication # 00078-06-E-01-U.

Warranty

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