Product Manual

StemTAG™ Alkaline Phosphatase Staining Kit (Red)

Catalog Number

CBA-300	100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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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 Staining Kit provides an efficient system for monitoring ES cell undifferentiation/ differentiation through AP activity by immunocytochemistry staining.

Related Products

1. CBA-301: StemTAG™ Alkaline Phosphatase Activity Assay Kit (Colorimetric)
2. CBA-306: StemTAG™ Alkaline Phosphatase Staining Kit (Purple)
3. CBA-312: MEF Feeder Cells (Puromycin-resistant)
4. CBA-316: SNL Feeder Cells
5. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

Kit Components

1. Fixing Solution (Part No. 30001): One bottle – 50 mL

2. StemTAG™ AP Staining Solution A (Part No. 30002): One amber bottle – 20 mL
3. StemTAG™ AP Staining Solution B (Part No. 30003): One amber bottle – 20 mL

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

Storage

Store all components at 4ºC.

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.

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**

Table 2. Preparation of StemTAG™ AP Staining Solution

Assay Protocol (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 4ºC. For long-term storage, overlay the cells with 1X PBS containing 20% Glycerol. Store at 4ºC.
8. Count the red stained cell colonies (undifferentiated ES cells) vs. colorless colonies (differentiated ES cells) using a light microscope.

Example of Results

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

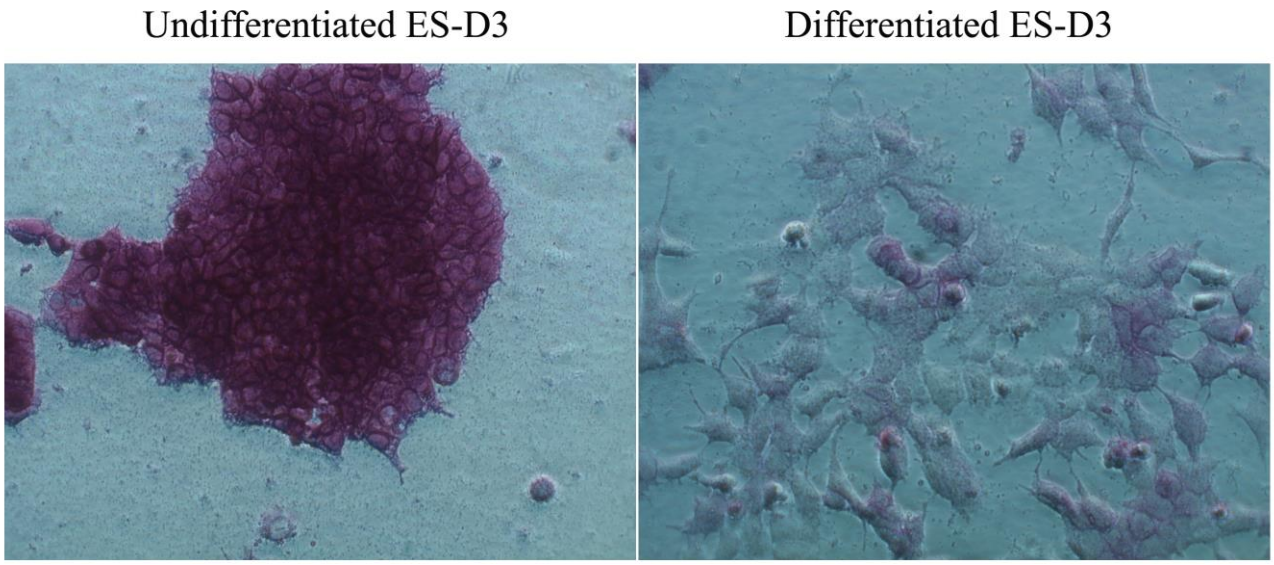

Figure 1: AP staining of ES Cells. Murine embryonic stem cells (ES-D3) are maintained in an undifferentiated stage on gelatin-coated dishes in the presence of LIF, as indicated by the high AP activity. To induce differentiation, LIF was withdrawn over a period of several days; various differentiation events were observed (cells became flattened and enlarged with reduced proliferation).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

References

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Recent Product Citations

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