

---

Product Manual

# StemTAG™ Alkaline Phosphatase Staining Kit (Purple)

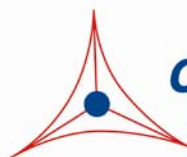
Catalog Number

CBA- 306

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.

<b>Marker Name</b>	<b>Mouse ES Cells</b>	<b>Mouse EG Cells</b>	<b>Human ES Cells</b>	<b>Human EG Cells</b>	<b>Human EC Cells</b>
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-300: StemTAG™ Alkaline Phosphatase Staining Kit (Red)
2. CBA-301: StemTAG™ Alkaline Phosphatase Activity Assay Kit (Colorimetric)
3. CBA-302: StemTAG™ Alkaline Phosphatase Staining and Activity Assay Kit (Colorimetric)
4. CBA-307: StemTAG™ Alkaline Phosphatase Activity Assay Kit (Fluorometric)
5. CBA-308: StemTAG™ Alkaline Phosphatase Staining and Activity Assay Kit (Fluorometric)
6. CBA-315: JK1 Feeder Cells

7. CBA-312: MEF Feeder Cells (Puromycin-resistant)
8. CBA-316: SNL Feeder Cells
9. CBA-325: StemTAG™ Stem Cell Colony Formation Assay
10. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

### **Kit Components**

1. Fixing Solution (Part No. C30001): One bottle – 50 mL
2. StemTAG™ AP Staining Solution (Part No. 130601): One amber bottle – 40 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**

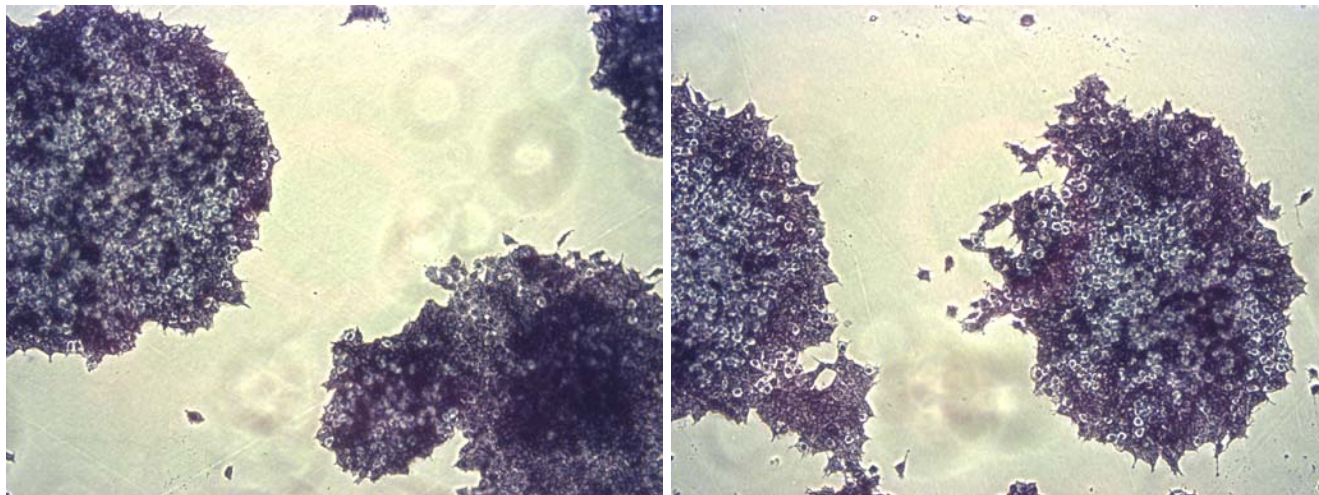
Store all components at 4°C.

### **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 StemTAG™ AP Staining Solution
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 purple 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 (Purple). 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. AP staining of undifferentiated ES cells was performed as described in the Assay Protocol.

## **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 Citation**

Khan, M.I. et al. (2016). Comparative gene expression profiling of primary and metastatic renal cell carcinoma stem cell-like cancer cells. *PLoS One* **11**:e0165718.

## **Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

## **Contact Information**

Cell Biolabs, Inc.  
7758 Arjons Drive  
San Diego, CA 92126  
Worldwide: +1 858-271-6500  
USA Toll-Free: 1-888-CBL-0505  
E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

©2012-2016: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.