
Product Manual

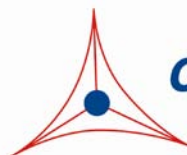
StemTAG™ Alkaline Phosphatase Staining and Activity Assay Kit (Fluorometric)

Catalog Number

CBA-308

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.

| 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 and Activity Assay Kit (Fluorometric) provides an efficient system for monitoring ES cell undifferentiation/ differentiation through AP activity by both immunocytochemistry staining and quantitative activity assay.. The fluorometric alkaline phosphatase activity assay is 50 times more sensitive than that obtained with the chromogenic substrate (see CBA-301).

Related Products

1. CBA-300: StemTAG™ Alkaline Phosphatase Staining Kit
2. CBA-301: StemTAG™ Alkaline Phosphatase Activity Assay Kit (Colorimetric)
3. CBA-302: StemTAG™ Alkaline Phosphatase Staining and Activity Assay Kit (Colorimetric)
4. CBA-308: StemTAG™ Alkaline Phosphatase Staining and Activity Assay Kit (Fluorometric)
5. CBA-315: JK1 Feeder Cells

6. CBA-312: MEF Feeder Cells (Puromycin-resistant)
7. CBA-316: SNL Feeder Cells
8. CBA-325: StemTAG™ Stem Cell Colony Formation Assay
9. 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 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 Fluorometric Substrate (100X) (Part No. 130701): One amber tube – 50 µL in DMSO
5. Cell Lysis Buffer (Part No. C30005): One bottle – 20 mL
6. Stop Solution (Part No. 130702): One bottle – 10 mL
7. Reference Standard (Part No. 130703): One amber tube – 100 µL of 5 mM Sodium Fluorescein in PBS

Materials Not Supplied

1. Human or Mouse Embryonic Stem Cells and Culture Medium
2. 1X PBS
3. Deionized Water
4. 1X PBST (1X PBS containing 0.05% Tween-20)
5. Light Microscope
6. Fluorescence microplate reader equipped with a 480 nm excitation filter and 520 nm emission filter

Storage

Store StemTAG™ AP Fluorometric Substrate (100X) protected from light at -20°C. Store all other components at 4°C.

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.

- 1X Substrate Solution: Freshly prepare only enough for immediate applications. Prepare a 1X Substrate Solution by diluting the provided 100X stock 1:100 in deionized water. Keep the 1X Substrate Solution away from light.

Preparation of Standard Curve

1. Prepare a 1:5 dilution series of Reference Standards in the concentration range of 0 – 50 μ M by diluting the 5 mM Reference Standard stock in 1X PBS (see Table 2).

| Standard Tubes | Reference Standard (μ L) | 1XPBS (μ L) | Fluorescein (nM) |
|----------------|-------------------------------|------------------|------------------|
| 1 | 10 | 990 | 50,000 |
| 2 | 100 of Tube #1 | 400 | 10,000 |
| 3 | 100 of Tube #2 | 400 | 2,000 |
| 4 | 100 of Tube #3 | 400 | 400 |
| 5 | 100 of Tube #4 | 400 | 80 |
| 6 | 100 of Tube #5 | 400 | 16 |
| 7 | 100 of Tube #6 | 400 | 3.2 |
| 8 | 0 | 400 | 0 |

Table 3. Preparation of Fluorescein Reference Standards

2. Transfer 100 μ L of each reference standard to a 96-well plate suitable for fluorescence measurement. Add 50 μ L of Stop Solution and mix by placing the plate on an orbital plate shaker for 30 seconds.
3. Read the fluorescence with a fluorescence plate reader at 480 nm excitation /520 nm emission.

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 4°C, 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 suitable for fluorescence measurement. 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 1X Substrate Solution. Mix the reaction mixture thoroughly by pipetting to ensure homogeneity.
7. Immediately begin reading sample and blank wells with a fluorescent microplate reader with an excitation wavelength of 480nm and an emission wavelength of 520nm. Read the wells in increments between 1 and 5 minutes for a total of 30 minutes.

Note: To normalize data, the readings from samples must be subtracted from a blank sample (no enzyme).

8. (optional) If an end-point reading is desired, stop the reaction by adding 50 µL of Stop Solution and mixing by placing the plate on an orbital plate shaker for 30 seconds. Read the fluorescence with a fluorescence plate reader at 480 nm/520 nm.

Example of Results

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

Undifferentiated ES-D3



Differentiated ES-D3



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).

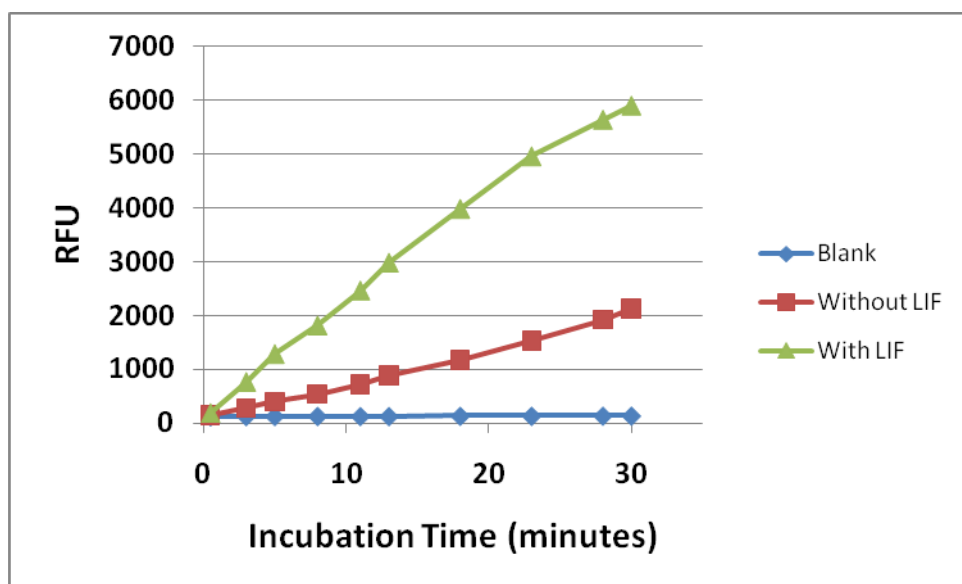
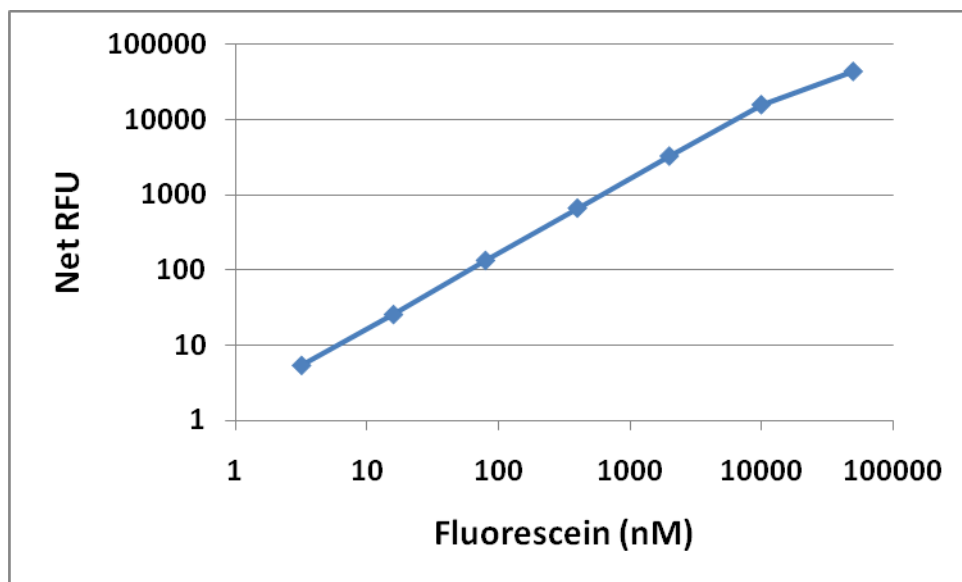


Figure 2: Fluorescein Reference Standard Curve and AP Activity Assay. **Top:** A serial 5-fold dilution of Fluorescein Reference Standards was prepared, and the fluorescence was read with a fluorescence plate reader at 480 nm/520 nm. **Bottom:** Mouse embryonic D3 cells were grown in the presence or absence of LIF for 5 days. Cell lysates were 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.

Warranty

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