

# Alkaline Phosphatase Activity Assay

Cat. No. 8258 500 tests

#### Introduction

Pluripotent stem cells (PSCs) have the unique capability to proliferate indefinitely and to differentiate into all cell types. Due to their self-renewal property and pluripotency, PSCs have become a promising candidate for therapeutic applications. Elevated level of alkaline phosphatase expression is one of the most widely used stem cell markers. ScienCell<sup>TM</sup> Alkaline Phosphatase Activity Assay is optimized to detect alkaline phosphatase activity in PSCs and provide a quantitative assay for measuring stem cell undifferentiation/differentiation.

## **Kit Components**

Cat. No.	# of vials	Name	Quantity	Storage
8258a	1	5× Substrate Stock	5 ml	-20°C, in the dark
8258b	1	Cell Lysis Buffer	25 ml	4°C
8258c	1	Stop Solution	25 ml	4°C
8258d	1	Assay Buffer	25 ml	4°C

#### **Procedures**

### A. Sample preparation

- 1. Gently aspirate the cell culture media from PSCs.
- 2. Wash PSCs twice with PBS. Aspirate the wash solution.
- 3. Lyse the cells with appropriate amount of Cell Lysis Buffer.
- 4. Centrifuge the cell lysate at 14,000 g for 5 min at 4°C. Collect the supernatant in a microcentrifuge tube.
- 5. Perform a protein assay to determine total protein concentration in lysate.
- 6. Dilute the cell lysate with Assay Buffer to a final volume of 50 μl per sample to obtain equal amount of total protein in each sample.

### B. Assay procedure

- 1. Dilute 5× Substrate Stock 1:5 in Assay Buffer to make 1× Substrate.
- 2. Apply 50  $\mu$ l of diluted cell lysate and 50  $\mu$ l of 1× Substrate to each well of the 96-well plate. Mix well and incubate for a desired period of time (10-60 minutes) at 37°C.
- 3. Stop the reaction by adding 50  $\mu$ l of Stop Solution to each well, mix well and measure the absorbance on an ELISA plate reader with a test wavelength at 405 nm.

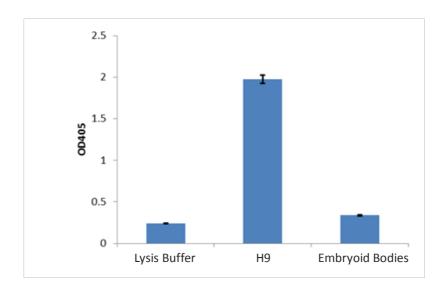


Figure 1. ScienCell<sup>TM</sup> Alkaline Phosphatase Activity Assay is applied to undifferentiated (H9) or differentiated (embryoid bodies) human embryonic stem cells. Twenty micrograms of cell lysate were incubated with 1× Substrate for 60 min, and then assayed for Alkaline Phosphatase activity.