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™ 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	-20ºC, in the dark
8258b	1	Cell Lysis Buffer	25 ml	4ºC
8258c	1	Stop Solution	25 ml	4ºC
8258d	1	Assay Buffer	25 ml	4ºC

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 4ºC. 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 µl of diluted cell lysate and 50 µl 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 37ºC.
3. Stop the reaction by adding 50 µl of Stop Solution to each well, mix well and measure the absorbance on an ELISA plate reader with a test wavelength at 405 nm.

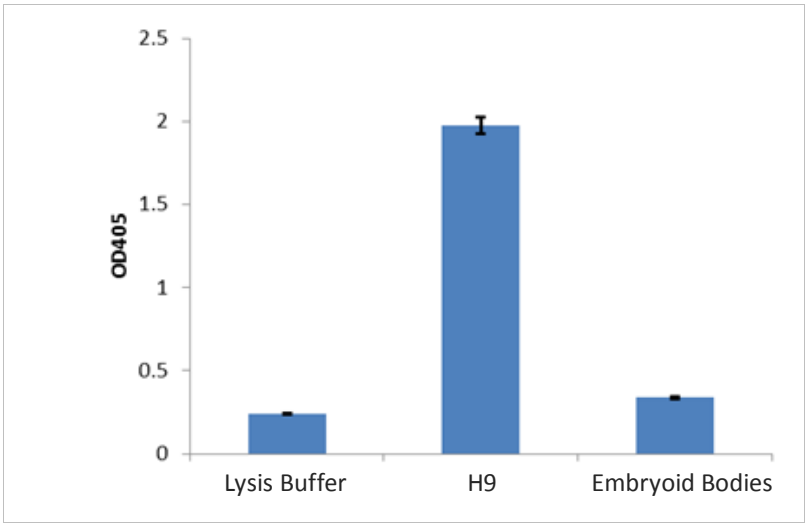

Figure 1. ScienCell™ Alkaline Phosphatase Activity Assay is applied to undifferentiated (H9) or differentiated (embryoid bodies) human embryonic stem cells. Twenty micrograms of cell lysate were incubated with 1× Substrate for 60 min, and then assayed for Alkaline Phosphatase activity.