Alkaline Phosphatase Activity Assay

(Using BD Clontech's SEAP Chemiluminescent Assay Kit)

(Ni Tang 01-22-08; updated by Bai-Cheng He 10-03-08; Commented by TCH)

REAGENTS AND BUFFER:

ALP Substrate: from BD Clontech, Cat# 631737.

1×LUPO Buffer:
(10mM Diethanolamine, 0.5mM MgCl2, 10mM L-Homoarginine, pH9.8)

1ml: **1M Diethanolamine** (Cat# D45-500, Fisher Scientific, stocks at room temperature; Original stock = 10.4M)

10ml: **100mM L-homoarginine Hydrochloride** (Cat#: AC169090010, Fisher Scientific, stocks at 4°C) (MW=224.69; for 100mM stock, 2.25g/100mL or 222.2mL/5g)

50ul: **1M MgCl2**

Adjust pH to 9.8, and add ddH2O to final volume of **100ml**.
Stocks are kept at -20°C in aliquots.

A. Preparation of Samples

Lyse the cells with 1x Luciferase Cell Lysis Buffer (from Promega **Cat# E1531, Cell Culture Lysis 5X Reagent**). For 24 wells plate, use 100ul per well. Wait for 5min till the cells are lyzed completely.

IMPORTANT NOTE: Other types of cell lysis buffer may significantly increase background reading. These include Promega's 5X Reporter Lysis Buffer and NEB's Cell Lysis Buffer, although BD Bioscience's Luciferase 3X Cell Lysis Buffer (Fisher Cat# NC9003365) yields acceptable background readings.

B. Chemiluminescent ALP Assay

1). **Prepare the ALP mixture:**

Sample Lysate	5ul
1×LUPO Buffer	15ul
ALP Substrate (BD Clontech, Cat# 631737)	5ul
Total	**25ul**

2). Mix well, and incubate at room temperature for **20-30min** (**NOTE**: Readings increase over time, up to 60min. However, background readings also increase if incubation is >30min. See the figure below).

3). Read the ALP activity by following the **Luciferase Assay procedure**.