Alkaline Phosphatase Activity Assay Kit (Colorimetric)

Alkaline Phosphatase Activity Assay Kit (Colorimetric) can be used to measure Alkaline Phosphatase activity in Human or Mouse cells.

Catalog number: ARG81239

Package: 100 assays

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INTRODUCTION

Alkaline phosphatase (ALP, ALKP, ALPase, Alk Phos) (EC 3.1.3.1), or basic phosphatase, is a homodimeric protein enzyme of 86 kilodaltons. Each monomer contains five cysteine residues, two zinc atoms and one magnesium atom crucial to its catalytic function, and it is optimally active at alkaline pH environments.

ALP has the physiological role of dephosphorylating compounds. The enzyme is found across a multitude of organisms, prokaryotes and eukaryotes alike, with the same general function but in different structural forms suitable to the environment they function in. Alkaline phosphatase is found in the periplasmic space of E. coli bacteria. This enzyme is heat stable and has its maximum activity at high pH. In humans, it is found in many forms depending on its origin within the body – it plays an integral role in metabolism within the liver and development within the skeleton. Due to its widespread prevalence in these areas, its concentration in the bloodstream is used by diagnosticians as a biomarker in helping determine diagnoses such as hepatitis or osteomalacia.

Undifferentiated pluripotent stem cells have elevated levels of alkaline phosphatase on their cell membrane, therefore alkaline phosphatase staining is used to detect these cells and to test pluripotency (i.e., embryonic stem cells or embryonal carcinoma cells). [Provide by Wikipedia: Alkaline phosphatase]

PRINCIPLE OF THE ASSAY

This Alkaline Phosphatase Activity Assay Kit employs a convenient colorimetric method for the detection of Alkaline Phosphatase from cell lysate samples (Mouse and Human embryonic stem cells). The samples, blank (Cell Lysis Buffer only) or AP Activity Assay Standards are added to a 96 well plate. Then, an AP Activity Assay Substrate is added into the wells and incubate. The color develops in proportion to the amount of Alkaline Phosphatase. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 405 nm. The concentration of Alkaline Phosphatase in the cell lysate samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
AP Activity Assay Substrate	5 mL (ready to use)	4°C
Cell Lysis Buffer	20 mL (ready to use)	4°C
10X Stop Solution	10 mL	4°C
AP Activity Assay Standards (5 mM p-Nitrophenol)	1 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 405 nm
- Light microscope
- Deionized or Distilled water
- 96 well plate
- 1X PBS
- 1X PBST (1X PBS containing 0.05% Tween-20)
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- All materials should be equilibrated to room temperature (RT, 20-25˚C) before use.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell lysate:

1. Culture mouse ES cells in medium containing Leukemia inhibitory factor (LIF); alternatively, culture human ES cells on a mouse embryonic fibroblasts (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. Incubate for 10 minutes at 4°C, remove the solution and spin down the cell debris at 12,000 x g for 10 minutes. Collect the supernatant as cell lysate. Perform a BCA assay or other protein assay to determine the protein concentration of the cell lysate.

REAGENT PREPARATION

- 1X Stop Solution: Dilute 10X Stop Solution into distilled water to yield 1X Stop Solution. (E.g., add 5 mL of 10X Stop Solution into 45 mL of distilled water to a final volume of 50 mL) Store 1X Stop Solution at room temperature.
- AP Activity Assay Standards: Dilute AP Activity Assay Standards 1:10 with Stop Solution to yield working Standards. (E.g., add 100 µL of AP Activity Assay Standard (5 mM pNP) into 900 µL of 1X Stop Solution to a final volume of 1 mL)

Dilute working Standards as according to the table below:

Standard tube	p-Nitrophenol conc. (µM)	Volume of 1X Stop Solution (µL)	Volume of working Standards (µL)
S1	250	500	500 of working standards
S2	125	500	500 of S1
S3	62.5	500	500 of S2
S4	31.3	500	500 of S3
S5	15.6	500	500 of S4
S6	7.8	500	500 of S5
S7	3.9	500	500 of S6
S8	1.95	500	500 of S7
S9	1	500	500 of S8
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and samples should be assayed in duplicates or triplicate.

1. Add **50 µL** of **Blank (Cell Lysis Buffer)** and **cell lysate samples** into the appropriate wells of 96-Well Microplate.
2. Initiate the reaction by adding **50 µL** of **AP Activity Assay Substrate**. Incubate for **10-30 minutes** at **37°C**.
3. Stop the reaction by adding **50 µL** of **1X Stop Solution** and mix by placing the plate on an orbital plate shaker for **30 seconds**.
4. Transfer **150 µL** of each dilution, in duplicate of **each serial diluted AP Activity Assay Standards**, to a 96-well plate. **(See REAGENT PREPARATION)**
5. Read the absorbance at **O.D. 405 nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)

EXAMPLE OF TYPICAL STANDARD CURVE

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