Signosis, Inc.
Innovative Plate Assay Solutions

ALP Activity Assay Kit

Catalog # EA-7093 **(For Research Use Only)**

Introduction

Alkaline phosphatase (ALP) is an important enzyme that is involved in removing phosphate groups from molecules during metabolic processes. This enzyme functions best in alkaline conditions and is found in several tissues throughout the body, including the liver, bones, kidneys, and intestines. ALP plays a key role in bone formation and the transport of metabolites across cell membranes. Because ALP is released into the bloodstream during liver or bone disorders, it is a clinically significant biomarker used to evaluate liver function and bone health. Elevated ALP levels are commonly associated with conditions such as bile duct obstruction, liver disease, and bone disorders including Paget's disease and osteomalacia.

Principle of the assay

The ALP assay kit utilizes a chromogenic substrate, p-nitrophenyl phosphate (pNPP), that releases a colored product when it hydrolyzed into pNP by ALP. Under alkaline conditions, pNP becomes yellow and can be measured spectrophotometrically at an absorbance of 405 nm.

Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading or 96-well black microplate with clear bottom for fluorescence reading
- Microplate reader capable of measuring absorbance at 560 nm or fluorescence at 530nm/590nm

Materials Provided

- pNPP Substrate (-20ºC)

****Spin down small tubes before starting experiment. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.
6. Use PBS to dilute the cell sample to the appropriate concentration, if necessary.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use PBS to dilute the tissue sample to the appropriate concentration, if necessary.

ALP Measurement

1. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
pNPP Substrate	1 µL
PBS	49 µL
Total	50 µL

2. Add 50 µL of reaction mix to each well of the plate.
3. Add 50 µL of sample to each well with reaction mix and mix thoroughly.
4. Cover the plate and incubate at room temperature for 10 minutes away from light.
5. Measure the absorbance of the plate in a plate reader at 405 nm.