BIOMEDICAL RESEARCH SERVICE CENTER
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Alkaline Phosphatase (ALP) Assay Kit (Cat #: E-102)

COMPONENTS: ALP Assay Solution- 40 ml (for 400 assays); **store in aliquots at -80°C after first thawing**
10x Cell Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**

PRODUCT DESCRIPTION: Alkaline phosphatase (ALP or ALK) possesses a hydrolytic activity removing phosphate groups from protein and non-protein molecules. The assay kit is based on conversion of p-nitrophenol phosphate to nitrophenol in an alkaline buffer. Nitrophenol exhibits an absorption maximum at 405 nm (molar extinction coefficient = 18.75 $mM^{-1}cm^{-1}$), allowing for sensitive detection of ALP activity in crude cell/tissue extracts, serum/plasma and urine. Assay solution is stable for several years if stored and handled properly.

Plasma: Plasma samples may need to be diluted with ice-cold 1x Cell Lysis Solution (from 10-fold dilution of 10x Cell Lysis Solution with dH_2O) to obtain assay linearity. Samples should be store at -80ºC.

Preparation of cell/tissue extracts:

1. Wash $10^5 - 10^6$ cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80ºC until use.
2. Add 50 – 100 µl ice-cold 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. For tissue extraction, weigh ~25 mg tissue and homogenize in 0.5 ml ice-cold 1x Cell Lysis Solution. Briefly spin homogenate at ~2,000 rpm for 5 sec in a microfuge to deposit insoluble tissue debris. The lysate can be subjected to a high-speed centrifugation to separate the membrane and cytosolic protein fractions if necessary. Store lysate at -80ºC.
3. Perform protein assay to determine sample protein concentration. Equalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution. A suggested protein concentration range is 0.2 – 1 mg/ml.

Enzyme assay for clear sample: Thaw ALP Assay Solution and keep solution on ice prior to assay.

1. Add 10 µl sample to each well of a 96-well plate. Add 10 µl dH_2O to a well serving as blank.
2. Reaction is initiated by adding 100 µl ALP Assay Solution to each well. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 30 min or 60 min.
3. Stop reaction by adding 20 µl 1N NaOH (not included in the kit) to each well followed by brief gentle agitation. Yellowish reaction color appears in wells during incubation.
4. Measure $O.D._{405\ nm}$ using a plate reader. Subtract blank well reading from each sample well reading to generate **O.D.**. For 30 min incubation, ALP enzyme activity in IU/L unit= µmol/(L•min)= **O.D.** x 1000 x 130 µl/(30 min x 0.6 cm x 18.75 x 10 µl)= **O.D. x 38.52**. For 60 min incubation, ALP activity in IU/L unit **= O.D. x 19.26.**

Enzyme assay for hemolyzed sample:

1. Add 10 µl sample to each well of a 96-well plate in duplicate.
2. Add 100 µl dH_2O to one set of sample wells. Add 100 µl ALP Assay Solution to the other set of sample wells. Mix contents by brief agitation. Cover plate and incubate at 37°C for 30 min or 60 min.
3. Stop reaction by adding 20 µl 1N NaOH (not included in the kit) to each dH_2O well and assay well followed by brief agitation.
4. Measure $O.D._{405\ nm}$ using a plate reader. Subtract dH_2O well reading from assay well reading for each sample to generate **O.D.**. For 30 min incubation, ALP enzyme activity in IU/L unit= µmol/(L•min)= **O.D.** x 1000 x 130 µl/(30 min x 0.6 cm x 18.75 x 10 µl)= **O.D. x 38.52**. For 60 min incubation, ALP activity in IU/L unit **= O.D. x 19.26.**

Additional information:

- Note that "0.6 cm" in the equation is the typical light path in a 96-well plate and may be custom adjusted as needed.
- A 1N NaOH solution needs to be prepared for reaction termination. Avoid skin contact with NaOH. Please refer to the product page of our website or contact us for MSDS information.