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

COMPONENTS: ALP Assay Solution- 20 ml (for 200 wells); store at -20°C
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

PRODUCT DESCRIPTION: 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 $mM^{-1}cm^{-1}$), allowing for sensitive detection of ALP activity in cell/tissue extracts and biological fluids such as serum/plasma. Assay solution is stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up at least ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 2 mg/ml.

Enzyme assay for clear sample:

1. Thaw ALP Assay Solution and keep solution on ice. Add 10 µl of each sample to a plain (uncoated) 96-well plate. Add 10 µl 1x Cell Lysis Solution or dH_2O to an empty 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 (do not use CO_2 incubator).
3. Stop reaction by adding 20 µl 1N NaOH (not included in the kit) to each well followed by brief gentle agitation.
4. Measure $O.D._{405\ nm}$ using a plate reader. Subtract blank well reading from each sample well reading to generate **ΔO.D.**.

Enzyme assay for colored sample (serum/plasma):

1. Thaw ALP Assay Solution and keep solution on ice. Add 10 µl of each sample to a plain (uncoated) 96-well plate <u>in duplicate</u> (for control set and reaction set).
2. Add 100 µl ice-cold dH_2O to one set of sample wells (control wells). Add 100 µl ALP Assay Solution to the other set of sample wells (reaction wells). Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 1 hour or 2 hours (do not use CO_2 incubator).
3. Stop assay by adding 20 µl 1N NaOH (not included in the kit) to each control well and reaction well followed by brief agitation.
4. Measure $O.D._{405\ nm}$ using a plate reader. Subtract control well reading from reaction well reading to generate **ΔO.D.** for each sample.

Enzyme activity calculation:

For 1-hour incubation, ALP enzyme activity in IU/L unit = µmol/(L•min) = **ΔO.D.** x 1000 x 130 µl / (30 min x 0.6 cm x 18 x 10 µl) = **ΔO.D. x 40.12**. For 2-hour incubation, ALP activity **= ΔO.D. x 20.06**. Sample enzyme activity can be presented as units/µg proteins.

Additional information:

- A solution of 1N NaOH 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.