BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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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 room temperature **(agitate vial to redissolve contents)**

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 measurement 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 dH_2O. Bring up ~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 microfuge at ~14,000 rpm at 4℃ for 5 min. Supernatant (lysate) 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 0.2 – 4 mg/ml. *Normalization of lysate protein concentration is recommended.*

Enzyme assay for clear sample:

1. Thaw ALP Assay Solution and keep solution on ice. *Do not over thaw solution.* Add 20 µl of each sample to a plain (uncoated) 96-well plate. Add 20 µl 1x Cell Lysis Solution 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. Measure $O.D._{405\ nm}$ using a plate reader. Subtract blank well reading from each sample well reading to generate **ΔO.D.** (see below for calculation of ALP activity).

Enzyme assay for colored sample:

1. Thaw ALP Assay Solution and keep solution on ice. *Do not over thaw solution.* Add 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control and reaction).

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 30 min or 60 min. *Do not use CO_2 incubator.*

3. 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 30-min incubation, enzyme activity in IU/L unit = µmol/(L•min) = **ΔO.D.** x 1000 x 120 µl / (30 min x 0.6 cm x 18 x 20 µl) = **ΔO.D. x 18.52**. For 60-min incubation, ALP activity **= ΔO.D. x 9.26**. Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.