Acid Phosphatase Assay Buffer:

Acid Phosphatase activity determination in biological samples (e.g. plasma, serum, cell lysate, tissue samples.)

**KIT CONTENTS (100 TESTS IN 96-WELL PLATES)**

**Assay Buffer:** 12 mL

**pNPP Liquid:** 280 µL

**Stop Reagent:** 12 mL

**Standard:** 1 mL

**Storage conditions:** The kit is shipped at room temperature. Store the Standard and stop reagent at 4°C and all other reagents at -20°C. Shelf life: 6 months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

**PROCEDURES**

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and Stop Reagent to samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

**Sample Preparation:** Serum and plasma should be diluted 2-5 fold.

**Tissue:** Prior to dissection, rinse tissue in Tris buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL 50 mM Tris buffer (pH 7.5). Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

**Cell Lysate:** Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold 50 mM Tris buffer (pH 7.5), approximately one million cells per mL. Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

All samples can be stored at –80 to –20°C for at least one month.

**Reagent Preparation:** Equilibrate all components to desired reaction temperature (e.g. 25°C or 37°C).

**Standard Preparation:**

Mix 20 µL of 12.5 mM Nitrophenol standard with 230 µL of dH2O to make 1000 µM Premix.

<table>
<thead>
<tr>
<th>No</th>
<th>Premix + dH2O</th>
<th>Vol (µL)</th>
<th>Nitrophenol (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 µL + 0 µL</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>60 µL + 40 µL</td>
<td>100</td>
<td>600</td>
</tr>
<tr>
<td>3</td>
<td>30 µL + 70 µL</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>0 µL + 100 µL</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**Reaction Preparation:**

1. Transfer 20 µL of each sample into separate wells. Transfer 20 µL of each standard (OD_STD) into wells of a clear flat bottom 96-well plate.
2. The Working Reagent is prepared by mixing together for each well 85 µL of assay buffer and 2 µL of pNP Liquid. Add 80 µL of Working Reagent to all standard and sample wells. Tap plate briefly to mix.
3. Incubate at 25°C or desired temperature for 30 minutes. Add 50 µL of Stop Reagent to each well. Tap plate briefly to mix.
4. Read OD405nm.

**Calculation**

Subtract blank OD (water, #4) from the standard OD values and plot the ΔOD against standard concentrations. Determine the Slope and use the following equation to calculate Acid Phosphatase activity.

\[
\text{ACP Activity} = \left( \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Time} \times \text{Slope}} \right) \times n \quad (U/L)
\]

where OD_SAMPLE is the OD405nm value for each sample and OD_BLANK is the OD405nm value of the water (standard #4) or the sample blank if one was used. Slope is the slope of the linear regression fit of the standard points and Time is the reaction time (30 min). n is the dilution factor.

**Unit definition:** 1 Unit (U) of ACP will catalyze the conversion of 1 µmole of p-Nitrophenyl phosphate to p-Nitrophenol and phosphate per min at 25°C and pH 5.3.

**Note:** If sample ACP activity exceeds 60 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with ACP activity < 1 U/L, the incubation time can be extended up to 60 minutes for greater sensitivity.

**MATERIALS REQUIRED, BUT NOT PROVIDED**

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

**LITERATURE**

