The alpha form of mannose.

Mix 5 µL of 12.5 mM Nitrophenol standard with 495 µL dH2O to make Standard Preparation:

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

Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

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 buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

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

**Standard Preparation:**

Mix 5 µL of 12.5 mM Nitrophenol standard with 495 µL dH2O to make 125 µM standard.

<table>
<thead>
<tr>
<th>No</th>
<th>125 µM STD + dH2O</th>
<th>Vol (µL)</th>
<th>Nitrophenol (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250 µL + 0 µL</td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>2</td>
<td>150 µL + 100 µL</td>
<td>250</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>50 µL + 200 µL</td>
<td>250</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>0 µL + 250 µL</td>
<td>250</td>
<td>0</td>
</tr>
</tbody>
</table>

**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 can be assayed directly.

**Tissue:** Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 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 buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

**APPLICATIONS**

α-Mannosidase activity determination in biological samples (e.g. plasma, serum, tissue and culture media.)

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

**Substrate:** 10 mL

**Stop Reagent:** 12 mL

**Standard:** 1 mL 12.5 mM Nitrophenol

**Storage conditions:** The kit is shipped at room temperature. Store all components at 4°C upon receiving. 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.

**REACTION PREPARATION**

1. Transfer 200 µL of each standard (OD STD) into wells of a clear flat bottom 96-well plate. Do not add anything else to the standard wells.
2. Transfer 10 µL of each sample into separate wells. Add 90 µL Substrate to each sample well. Tap plate briefly to mix.
3. Incubate at 25°C or room temperature for 10 minutes. Add 100 µL of Stop Reagent to each sample well. Tap plate briefly to mix.
4. Read OD 405 nm.

**Note:** If your sample is colored or opaque, then a sample blank (OD BLANK) will be needed. Add 10 µL of sample to a well, and add 90 µL of dH2O. After incubation add 100 µL Stop Reagent.

**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 α-Mannosidase activity.

\[
\text{AMA Activity} = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Time} \times \text{Slope}} \times \frac{\text{Reaction Vol (µL)}}{\text{Sample Vol (µL)}} \times n \ (U/L)
\]

where \(\text{OD}_{\text{SAMPLE}}\) is the OD 405 nm value for each sample and \(\text{OD}_{\text{BLANK}}\) is the OD 405 nm 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 (10 min). Reaction Vol and Sample Vol are 200 µL and 10 µL, respectively. \(n\) is the dilution factor.

**Unit definition:** 1 Unit (U) of AMA will catalyze the conversion of 1 umole of 4-Nitrophenyl-α-D-mannopyranoside to 4-Nitrophenol and α-D-Mannose per min at 25°C and pH 4.5.

Note: If sample AMA activity exceeds 250 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with AMA activity < 5 U/L, the incubation time can be extended up to 30 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