Alpha-Mannosidase (AMA) Assay Kit (Colorimetric)

LS-K195-100 (100 Tests) • **Store at -20°C**

Introduction

α-MANNOSIDASE (AMA) is an enzyme which catalyzes the cleavage of the alpha form of mannose. α-Mannosidase assists in the breakdown of complex sugars from glycoproteins in the lysosome. Defective AMA or deficient AMA activity causes α-mannosidosis and leads to deterioration of the central nervous system in children. This non-radioactive, colorimetric AMA assay is based on the cleavage of 4-nitrophenol from the synthetic substrate. Nitrophenol becomes intensely colored after addition of the stop reagent. The increase in absorbance at 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

Key Features

- Fast and sensitive. Linear detection range (10 µL sample): 1 to 250 U/L for a 10 minute reaction.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

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

Components

Component	K195-100
	100 Tests
Substrate	10 mL
Stop Reagent	12 mL
Standard (12.5 mM Nitrophenol)	1 mL

Materials Not Supplied

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.

Storage

The kit is shipped at room temperature. Store all components at 4°C upon receiving. Shelf life: 6 months after receipt.

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Assay Procedure

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.

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 dH_2O to make 125 µM standard.

No	125 µM STD + dH2O	Vol (µL)	Nitrophenol (µM)
1	250 µL + 0 µL	250	125
2	150 µL + 100 µL	250	75
3	50 µL + 200 µL	250	25
4	0 µL + 250 µL	250	0

Procedure

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_{405nm}.

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 dH_2O. After incubation add 100 µL Stop Reagent.

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Calculations

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.

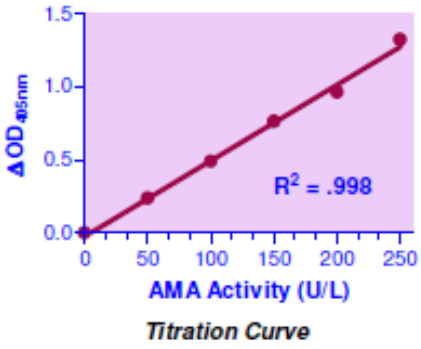
$$\text{AMA Activity} = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Time} \bullet \text{Slope}} \times \frac{\text{Reaction Vol (µL)}}{\text{Sample Vol (µL)}} \times n \ \text{(U/L)}$$

where OD_{SAMPLE} is the OD_{405nm} value for each sample and OD_{BLANK} is the OD_{405nm} 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.

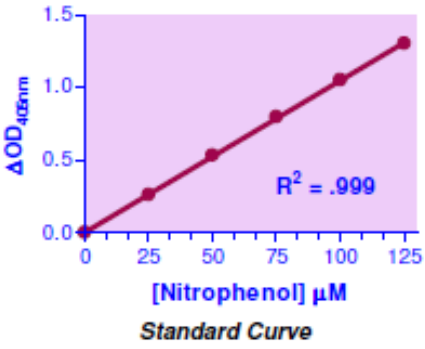
Unit definition: 1 Unit (U) of AMA will catalyze the conversion of 1 µmole of 4-Nitrophenyl-α-D-mannopyranoside to 4-Nitrophenol and α-DMannose per min at 25°C and pH 4.5.

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.

Sample Data



Titration Curve



Standard Curve

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