QuantiChrom™ α-Mannosidase Assay Kit (DAMA-100)

Quantitative Colorimetric Kinetic α-Mannosidase Activity Determination

DESCRIPTION

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. BioAssay Systems' 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.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Substrate Buffer: 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.

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.

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

Reagent Preparation: Equilibrate Substrate Buffer to desired reaction temperature (e.g. 25°C or 37°C).

Standard Preparation:

Mix 10 µL of 12.5 mM Nitrophenol standard with 490 µL dH_2O to make 250 µM standard.

No	250 µM STD + dH2O	Vol (µL)	Nitrophenol (µM)
1	200 µL + 0 µL	200	250
2	120 µL + 80 µL	200	150
3	60 µL + 140 µL	200	75
4	0 µL + 200 µL	200	0

Reaction Preparation:

1. Transfer 100 µL of each standard (OD_{STD}) into wells of a clear flat bottom 96-well plate.
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 desired temperature for 10 minutes. Add 100 µL of Stop Reagent to each 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.

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.

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

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times n \quad \text{(U/L)}$$

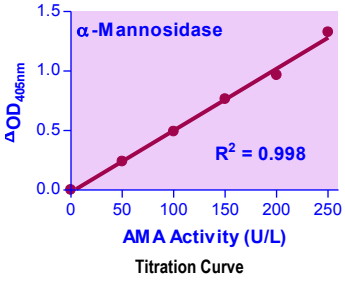
where OD_{SAMPLE} is the OD_{405nm} value for each sample and OD_{BLANK} is the OD_{405nm} value of the water (standard #4) or the sample blank if one was used. Slope is the slope of the linear regression fit of the standard points and Time is the reaction time (10 min). Reaction Vol and Sample Vol are 100 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-D-Mannose per min at 25°C and pH 4.5.

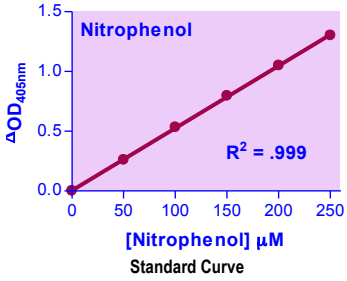
Note: If sample AMA activity exceeds 250 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with AMA activity < 5 U/L, the incubation time can be extended up to 30 minutes for greater sensitivity.

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.



Titration Curve



Standard Curve

LITERATURE

1. Rajesh, T et. al. (2013) Putative Role of a Streptomyces coelicolor-Derived α-Mannosidase in Deglycosylation and Antibiotic Production. Appl Biochem Biotechnol. [Epub ahead of print]
2. Nemčovičová, I et. al. (2013) Characterization of Class I and II α-Mannosidases from Drosophila melanogaster. Glycoconj J. 30(9):899-909.
3. Rangarajan, M et. al. (2013) Characterization of the α- and β-Mannosidases of Porphyromonas gingivalis. J Bacteriol. 195(23):5297-307.