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Product Information

α-Mannosidase Activity Assay Kit

Catalog Number **MAK318**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product 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.

The α-Mannosidase Activity Assay Kit provides a simple and high-throughput adaptable assay for determining α-Mannosidase activity in a variety of biological samples such as plasma, serum, tissue, and cell culture media. In this assay 4-nitrophenol is cleaved from a synthetic substrate. Nitrophenol becomes intensely colored with the addition of the stop reagent, producing a colorimetric (405 nm) result, directly proportional to the enzyme activity in the sample.

One unit of AMA is the amount of enzyme that will convert 1.0 μmole of 4-Nitrophenyl-α-D-manno-pyranoside to 4-Nitrophenol and α-D-Mannose per minute at 25 °C and pH 4.5. The kit has a linear detection range of 1–250 unit/L for a 10 minute reaction in a 96 well format.

Components

The kit is sufficient for 100 assays in 96 well plates.

Substrate Buffer Catalog Number MAK318A	10 mL
Stop Reagent Catalog Number MAK318B	12 mL
Nitrophenol Standard, 12.5 mM Catalog Number MAK318C	1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Substrate Buffer – Allow buffer to come to room temperature before use.

Nitrophenol Standard – Dilute 10 μL of 12.5 mM Nitrophenol standard with 490 μL of water to generate a 250 μM Standard Solution.

Storage/Stability

The kit is shipped at room temperature. Storage at 2–8 °C is recommended.

Procedure

All samples and standards should be run in duplicate. This assay is based on a kinetic reaction. Use of a multichannel pipettor is recommended to ensure identical incubation times. Addition of Substrate and Stop Reagent should be quick and mixing should be brief but thorough.

<u>Nitrophenol Standards for Colorimetric Detection</u>
Add 0, 60, 120, and 200 μL of the 250 μM Standard Working Solution into wells of a 96 well plate. Add water to each well to bring the volume to 200 μL, generating 0 (blank), 75, 150, and 250 μM standards.

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 the samples at 10,000 × *g* for 15 minutes at 2–8 °C. Remove supernatant for assay.

Cell Lysate – Collect the cells by centrifuging at 2,000 × *g* for 5 minutes at 2–8 °C. For adherent cells, it is recommended to use a rubber policeman. Do not harvest cells using proteolytic enzymes.

Homogenize cells in ice cold buffer containing 50 mM potassium phosphate, pH 7.5. Centrifuge at 10,000 × *g* for 15 minutes at 2–8 °C. Remove supernatant for assay.

All samples can be stored at –70 °C/–20 °C for at least one month.

Assay Reaction
1. Add 200 µL of each standard into appropriate wells of a 96 well plate.
2. Add 10 µL of each sample into separate wells. Add 90 µL of Substrate Buffer to each sample well. Tap plate briefly to mix. Incubate at 25 °C for 10 minutes.
3. Add 100 µL of Stop Reagent to each sample well (Do not add to standards). Tap plate briefly to mix.
4. Measure the absorbance at 405 nm (A_{405}).

Note: If sample is colored or opaque, then a sample blank should be prepared. Add 10 µL of sample to a well, and add 90 µL of ultrapure water. After incubation add 100 µL of Stop Reagent.

Results
Calculations
Note: A new standard curve must be set up each time the assay is run.

The background is the value obtained for the 0 (assay blank) Nitrophenol Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Nitrophenol standards to plot a standard curve. Determine the slope using linear regression fitting.

The α-Mannosidase activity of a sample may be determined by the following equation:

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

Reaction time in minutes
Reaction volume (µL)
Sample volume (µL)
n = Dilution factor

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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