alpha Mannosidase Activity Assay Kit (Colorimetric)

alpha Mannosidase Activity Assay Kit (Colorimetric) can be used to measure alpha Mannosidase activity in serum, plasma, tissue and cell culture supernatants.

Catalog number: ARG82182

Package: 100 tests

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INTRODUCTION

alpha-Mannosidase (EC 3.2.1.24, alpha-D-mannosidase, p-nitrophenyl-alpha-mannosidase, alpha-D-mannopyranosidase, 1,2-alpha-mannosidase, 1,2-alpha-D-mannosidase, exo-alpha-mannosidase) is an enzyme involved in the cleavage of the alpha form of mannose. Its systematic name is alpha-D-mannoside mannohydrolase. It can be utilized in experiments that determine the effects of the presence or absence of mannose on specific molecules, such as recombinant proteins that are used in vaccine development. [Provide by Wikipedia: alpha-Mannosidase]

PRINCIPLE OF THE ASSAY

This alpha Mannosidase Activity Assay Kit (Colorimetric) is a simple assay that measures the amount of alpha mannosidase (AMA) present in biological samples. This 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 O.D. 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Substrate Buffer	10 mL	4°C
Stop Reagent	12 mL	4°C
Standard (12.5 mM Nitrophenol)	1 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 405 nm
- Clear flat-bottom 96 well microplate
- Centrifuge and centrifuge tubes
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2,500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2,000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Tissue lysate: rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~250 µL of cold 50 mM potassium phosphate buffer, pH 7.5. Centrifuge samples at 10,000 x g for 15 minutes at 4°C. Collect supernatant for assay.

Cell lysate: collect cells by centrifugation at 2,000 x g for 5 minutes 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 minutes at 4°C. Collect supernatant for assay.

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

REAGENT PREPARATION

- **Standard:** Mix 10 µL of 12.5 mM Nitrophenol Standard with 490 µL of distilled water (final conc. 250 µM). Dilute Standards as follow:

Standard tube	Nitrophenol (µM)	Distilled water (µL)	Standard Premix, 250 µM (µL)
S1	250	0	200
S2	150	80	120
S3	75	140	60
S4	0	200	0

ASSAY PROCEDURE

Equilibrate reagents to desired reaction temperature (E.g., 25 or 37°C). Briefly centrifuge tubes before use.

	Standard well	Sample well
Each diluted Standard	100 µL	
Each Sample		10 µL
Substrate Buffer		90 µL
Tap plate to mix briefly. Incubate for **10 minutes** at **room temperature** or **37°C**.		
Stop Reagent	100 µL	100 µL
Tap plate to mix briefly. Read the absorbance at **O.D. 405 nm**.		

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

CALCULATION OF RESULTS

1. Subtract blank OD (distilled water, S4) 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.

   AMA Activity (U/L)

   = [(OD_{Sample} – OD_{Blank}) / (Time x Slope)] x (Reaction Vol / Sampe Vol) x n

   = [(OD_{Sample} – OD_{Blank}) / Slope] x n

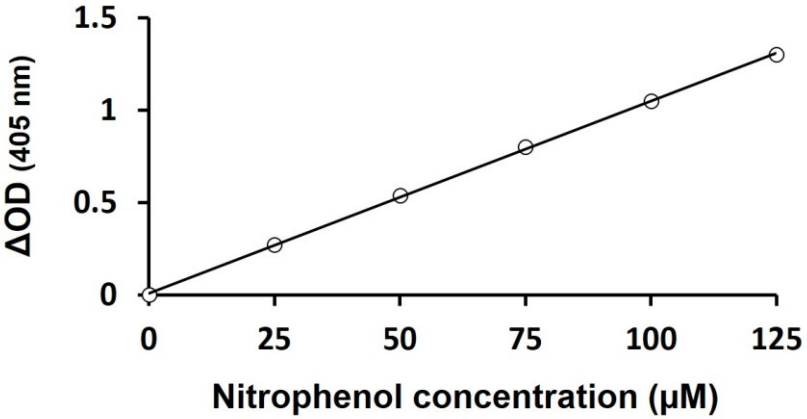
   Note:

   - OD_{Sample}, OD_{Blank}: the O.D. 405 nm values of the samples and distilled water (S4) or sample blank (if one was used).
   - Slope is the slope of the linear regression fit of the standard points.
   - Time is the reaction time (10 minutes).
   - Reaction Vol and Sample Vol are 100 µL and 10 µL, respectively.
   - n is the dilution factor.

2. 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 minute at 25°C and pH 4.5.
3. If sample AMA activity > 250 U/L, either use a shorter reaction time or dilute samples in distilled 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.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the alpha Mannosidase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

1 U/L