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ab272519

Alpha-Mannosidase

Assay Kit

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Alpha-Mannosidase Assay Kit datasheet:

www.abcam.com/ab272519

(use www.abcam.cn/ab272519 for China, or www.abcam.co.jp/ab272519 for Japan)

For quantitative determination of alpha-Mannosidase activity in biological samples.

This product is for research use only and is not intended for diagnostic use.

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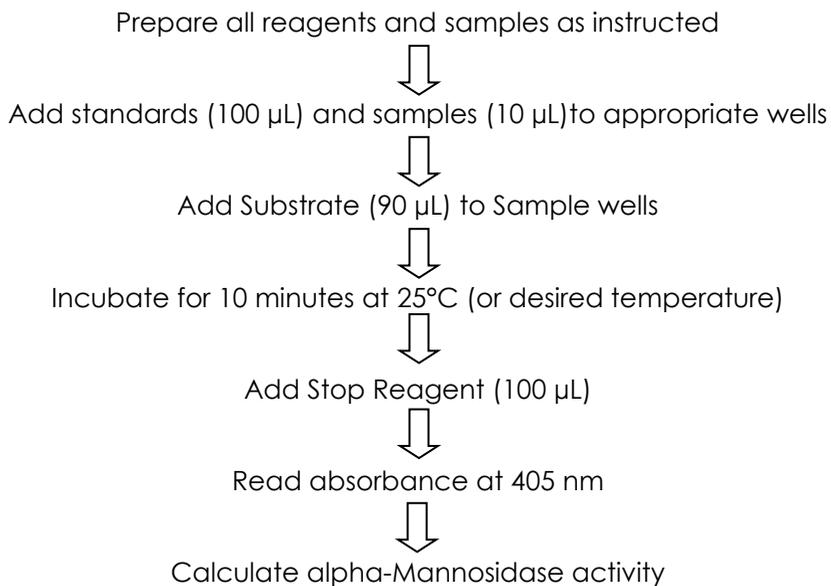
1. Overview

Alpha-Mannosidase Assay Kit (ab272519) is a non-radioactive, colorimetric alpha-Mannosidase assay is based on the cleavage of 4-nitrophenol from a 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.

Fast and sensitive: Linear detection range (10 μ L 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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 6 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Substrate Buffer	10 mL	+4°C
Stop reagent	12 mL	+4°C
Standard (12.5 mM Nitrophenol)	1 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 1X PBS
- 1.5 mL tubes
- 1.5 mL centrifuge
- Heat block or water bath
- 96-well clear plate with flat bottom
- Standard microplate reader - capable of reading absorbance at 405 nm

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (25°C or 37°C) prior to use. The kit contains enough reagents for 100 assays.

All reagents are supplied ready to use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

10.1 Mix 10 μL of Standard (12.5 mM Nitrophenol) with 490 μL dH₂O to make a 250 μM Standard.

10.2 Prepare standards as shown in the table.

Standard #	250 μM Nitrophenol (μL)	H ₂ O (μL)	Nitrophenol concentration. (μM)
1	200	-	250
2	120	80	150
3	60	140	75
4	-	200	0

11. Sample Preparation

11.1 Serum and plasma

11.1.1 These samples types can be assayed directly.

11.2 Tissue:

11.2.1 Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood.

11.2.2 Homogenize tissue (50 mg) in ~200 μ L buffer containing 50 mM potassium phosphate (pH 7.5).

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

11.3 Cell Lysate:

1.1.1 Collect cells by centrifugation at 2,000 x g for 5 minutes at 4°C.

Δ Note: For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman.

1.1.2 Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5).

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

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

12. Assay Procedure

- Equilibrate all prepared reagents to desired assay temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Reaction:

- 12.1** Transfer 100 μL of each standard (OD_{STD}) into wells of a clear flat bottom 96-well plate.
- 12.2** Transfer 10 μL of each sample into separate wells. Add 90 μL Substrate to each sample well. Tap plate briefly to mix.
- 12.3** Incubate at 25°C or desired temperature for 10 minutes.
- 12.4** Add 100 μL of Stop Reagent to each well. Tap plate briefly to mix.
- 12.5** Read $\text{OD}_{405\text{nm}}$.

Δ 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.

Δ Note: 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.

Δ Note: Use 96-well clear, flat-bottom plates.

13. Calculations

- 13.1** Subtract blank OD (water, Standard #4) from the standard OD values and plot the Δ OD against standard concentrations.
- 13.2** Determine the slope using linear regression fitting.
- 13.3** The alpha-Mannosidase activity concentration of the Sample is calculated as follows:

$$\begin{aligned} \text{AMA activity} &= \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Time} \times \text{Slope}} \times \frac{\text{Reaction volume } (\mu\text{L})}{\text{Sample volume } (\mu\text{L})} \times n \\ &= \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope}} \times n \text{ (U/L)} \end{aligned}$$

AMA = alpha-Mannosidase

OD_{Sample} = OD₄₀₅ value of the sample

OD_{Blank} = OD₄₀₅ value of water (Standard #4) or sample blank, if one is used

Slope = Linear regression fit of the Standard data points

Time = Reaction time (in minutes)

n = Dilution factor

Reaction volume = 100 μ L

Sample volume = 10 μ L

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

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

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

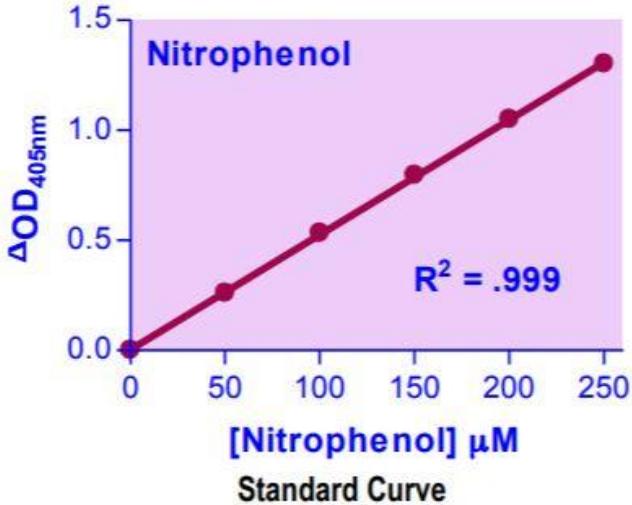


Figure 1. Example of 4-Nitrophenol standard curve.

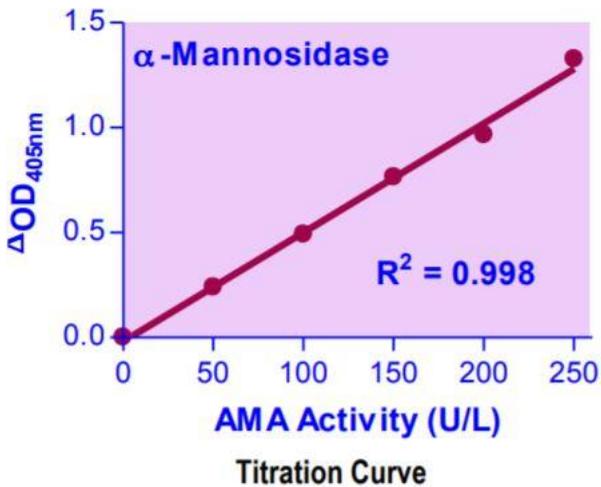


Figure 2: Example of alpha-Mannosidase Assay Kit activity titration.

15. Notes

Technical Support

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