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ab272525 a-L-Fucosidase Assay Kit

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a -L-Fucosidase Assay Kit datasheet:
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For quantitative determination of a-L-Fucosidase activity in biological samples..

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

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1. Overview

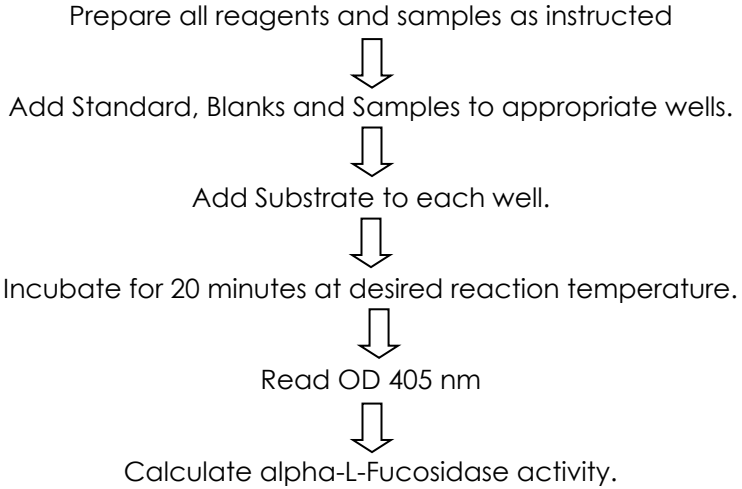
alpha-L-Fucosidase Assay Kit (ab272525) is a non-radioactive, colorimetric assay based on the cleavage of 4-nitrophenol from the synthetic substrate. Nitrophenol becomes intensely colored after addition of the stop reagent. The increase in absorbance at 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

High sensitivity and wide linear range. Linear detection range (10 µL sample): 1 to 100 U/L for a 20 minute reaction.

Homogeneous and simple procedure. Simple add-mix-read procedure allows reliable quantitation of fucosidase activity within 20 minutes.

Robust and amenable to HTS. All reagents are compatible with high throughput liquid handling instruments. Can be readily automated to measure 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, providing components have not been reconstituted.

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:

- Centrifuge tubes
- Pipetting devices and accessories
- 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 (18-25°C) prior to use.
- The kit contains enough reagents for 100 assays.

All reagents are supplied ready to use.

10. Standard Preparation

10.1 Mix 10 µL of Standard (12.5 mM Nitrophenol) with 490 µL dH_2O to make 250 µM Standard.

10.2 Use the 250 µM Standard to prepare the Standards detailed in the table.

Standard #	250 µM Standard	H_2O (µL)	Nitrophenol (µM)
1	200	0	250
2	120	80	150
3	60	140	75
4	0	200	0

11. Sample Preparation

11.1 Plasma and serum

11.1.1 Plasma and serum 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 µL 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:

11.3.1 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.

11.3.2 Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5).
11.3.3 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 prepared reagents to the desired reaction temperature (e.g. 25°C or 37°C) prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Reaction:

12.1 Transfer 100 µL Standard (OD_{STD}) into wells of a clear flat bottom 96-well plate.

12.2 Transfer 20 µL of each sample into separate wells. Add 80 µL Substrate to each sample well. Tap plate briefly to mix.

12.3 Incubate at 25°C or desired temperature for 20 minutes. Add 100 µL of Stop Reagent to each well. Tap plate briefly to mix.

12.4 Read OD405nm.

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: If the sample is colored or opaque, then a sample blank (OD_{BLANK}) will be needed. Add 20 µL of sample to a well, and add 80 µL of dH_2O. After incubation add 100 µL Stop Reagent.

13. Calculations

13.1 Subtract blank OD from the standard OD values and plot the ΔOD against standard concentrations. Determine the Slope and use the following equation to calculate a-Fucosidase activity (AFU):

$$\text{AFU activity} = \frac{\Delta OD_{SAMPLE} - \Delta OD_{BLANK}}{\text{time} \times \text{slope}} \times \frac{\text{Reaction volume (µL)}}{\text{Sample volume (µL)}} \times n$$

$$= \frac{1}{4} \times \frac{\Delta OD_{SAMPLE} - \Delta OD_{BLANK}}{\text{slope}} \times n \quad \text{(U/L)}$$

OD_{SAMPLE} = OD405nm of each Sample
OD_{BLANK} = OD405nm value of the water (or the Sample Blank if one is used)
Slope = the linear regression fit of the standard points and
Time = reaction time (20 min).
Reaction vol = 100 µL
Sample vol = 20 µL
n = Dilution factor

Unit definition: 1 Unit (U) of AFU will catalyze the conversion of 1 µmole of 4-Nitrophenyl-a-L-fucopyranoside to 4-Nitrophenol and a-L-Fucose per min at 25°C and pH 5.3.

Δ Note: If the sample AFU activity exceeds 100 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay.

Δ Note: For samples with AFU activity < 5 U/L, the incubation time can be extended up to 40 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.

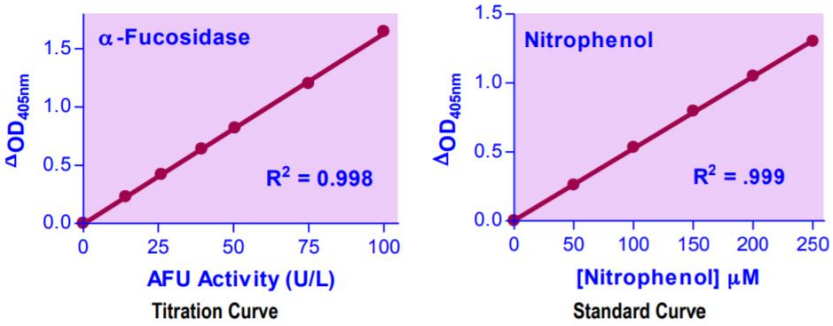


Figure 1. Example of a-L-Fucosidase titration curves.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)