alpha-L-fucosidase Activity Assay Kit (Colorimetric)

alpha-L-fucosidase Activity Assay Kit (Colorimetric) can be used to measure alpha-L-fucosidase activity in serum, plasma, tissue and cell lysate.

Catalog number: ARG82157

Package: 100 tests

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INTRODUCTION

In enzymology, an alpha-L-fucosidase (EC 3.2.1.51) is an enzyme that catalyzes the chemical reaction

alpha-L-fucoside + H_2O ⟺ L-fucose + alcohol

This enzyme belongs to the family of hydrolases, specifically those glycosidases that hydrolyse O- and S-glycosyl compounds. The systematic name of this enzyme class is alpha-L-fucoside fucohydrolase. This enzyme is also called alpha-fucosidase. This enzyme participates in n-glycan degradation and glycan structures- degradation.

Deficiency of this enzyme is called Fucosidosis. [Provide by Wikipedia: Alpha-L-fucosidase]

PRINCIPLE OF THE ASSAY

This alpha-L-fucosidase Activity Assay Kit (Colorimetric) is a simple assay that measures the amount of alpha-L-fucosidase (AFU) 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 with blue ice. Store all components at -20°C upon receiving.

Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Substrate Buffer	10 mL	-20°C
Stop Reagent	12 mL	-20°C
Standard (12.5 mM Nitrophenol)	1 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 405 nm
- Centrifuge and centrifuge tube
- Clear flat-bottom 96 well microplate
- Deionized or distilled water
- Cold buffer containing 50 mM potassium phosphate (pH 7.5)
- 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.
- Equilibrate Substrate Buffer to desired reaction temperature (e.g. 25°C or 37°C).
- 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.
- 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 pipette is recommended.
- 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 1500 x g for 15 minutes at 4°C. Collect the serum and assay directly. Assay sample immediately or aliquot & store samples at -20°C for at least 1 month. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood with heparin or citrate and centrifuge at 1500 x g for 15 minutes at 4°C. Collect the plasma layer and assay directly. Assay sample immediately or aliquot & store samples at -20°C for at least 1 month. Avoid repeated freeze-thaw cycles.

Tissue lysate: Prior to dissection, rinse tissue in PBS (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 X g for 15 min at 4°C. Collect supernatant for assay. Assay sample immediately or aliquot & store samples at -20°C for at least 1 month. Avoid repeated freeze-thaw cycles.

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. Collect supernatant for assay. Assay sample immediately or aliquot & store samples at -20°C for at least 1 month. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Standards:** Mix 10 µL of 12.5 mM nitrophenol standard with 490 µL of distilled water to make 250 µM standard premix. Prepare standards as follows,

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

	Standard wells	Sample wells
Standards	100 µL	
Sample		20 µL
Substrate		80 µL
Tap plate to mix well. Incubate for **20 minutes** at **desired temperature. (25°C or 37°C)**		
Stop Reagent	100 µL	100 µL
Tap plate to mix well. Read the absorbance at **O.D. 405 nm.**		

Note: If your 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 distilled water. After incubation add 100 µL 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 alpha-L-Fucosidase activity:

   AFU Activity (U/L)

   = $[(OD_{Sampe} - OD_{Blank}) / (Slope \times t)] \times (Reaction\ Vol / Sample\ Vol) \times n$

   = $[(OD_{Sampe} - OD_{Blank}) / Slope] \times 0.25 \times n$

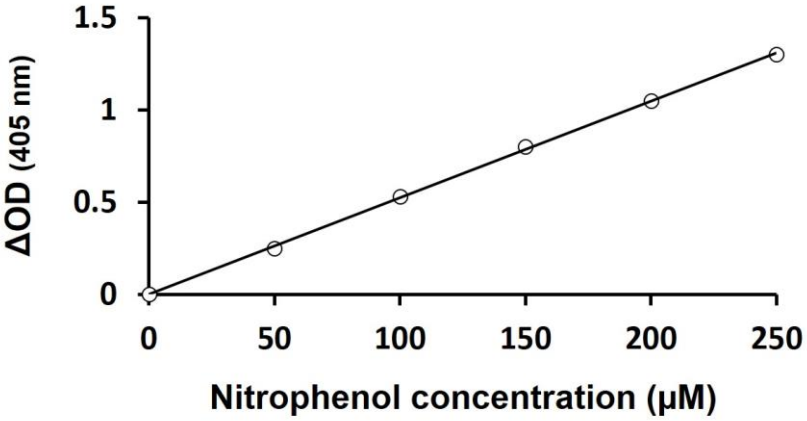
   Note:

   - OD_{Sampe}: the O.D. 405 nm value of each sample.
   - OD_{Blank}: the O.D. 405 nm value of blank (S4) or sample blank.
   - Slope: Slope is the slope of the linear regression fit of the standard points.
   - t: t is the incubation time (20 minutes)
   - n: n is the dilution factor.
   - Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively.
   - If Sample blank is used, use $OD_{Sampe\ blank}$ instead of OD_{Blank}.
2. arigo provides GainData®, an in-house development ELISA data calculator, for slope analysis. The slope data can easily be assayed when select regression model as “linear” in GainData®. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
3. If sample AFU activity exceeds 100 U/L, either use a shorter reaction time or dilute samples in distilled water and repeat the assay. For samples with AFU activity < 5 U/L, the incubation time can be extended up to 40 minutes for greater sensitivity.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the alpha-L-fucosidase 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