

α -L-Fucosidase (AFU) Assay Kit (Colorimetric)

LS-K247-100 (100 Tests) • Store at 4°C



Introduction

α -L-FUCOSIDASE (AFU) is an enzyme coded by the FUCA1 gene in humans and catalyzes the breakdown of L-Fucose. A genetic deficiency in this enzyme results in a neurovisceral storage disease, fucosidosis, which is characterized by the accumulation of fucose. Low serum activity of fucosidase has also been linked to ovarian carcinoma. Elevated fucosidase serum activity has been observed in patients with diabetes, hyperthyroidism, cirrhosis, and hepatitis. Increased activity has been associated with lung, breast, stomach, ovary, uterus, and liver carcinomas. This non-radioactive, colorimetric AFU 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 405 nm after addition of the stop reagent is directly proportional to the enzyme activity.

Key Features

- High sensitivity and wide linear range. Linear detection range (10 μ L sample): 1 to 100 U/L.
- 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.

Applications

- α -L-Fucosidase activity determination in biological samples (e.g. plasma, serum, tissue, cell lysate, etc.)

Components

Component	K247-100
	100 Tests
Substrate Buffer	10 mL
Stop Reagent	12 mL
Standard (12.5 mM Nitrophenol)	1 mL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

Storage

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

FOR RESEARCH USE ONLY! Not for use in humans.

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Assay Procedure

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.

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 μ L buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

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. Remove supernatant for assay.

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

Reagent Preparation

Equilibrate Substrate Buffer to desired reaction temperature (e.g. 25°C or 37°C).

Standard Preparation

Mix 10 μ L of 12.5 mM nitrophenol standard with 490 μ L dH₂O to make 250 μ M standard.

No	250 μ M STD + dH ₂ O	Vol (μ L)	Nitrophenol (μ M)
1	200 μ L + 0 μ L	200	250
2	120 μ L + 80 μ L	200	150
3	60 μ L + 140 μ L	200	75
4	0 μ L + 200 μ L	200	0

Procedure

1. Transfer 100 μ L of each standard (OD_{STD}) into wells of a clear flat-bottom 96-well plate.
2. Transfer 20 μ L of each sample into separate wells. Add 80 μ L Substrate to each sample well. Tap plate briefly to mix.
3. Incubate at 25°C or desired temperature for 20 minutes. Add 100 μ L of Stop Reagent to each well. Tap plate briefly to mix.
4. Read OD_{405nm}.

Note: If your sample is colored or opaque, then a sample blank (OD_{BLANK}) will be needed. Add 20 μ L of sample to a well, and add 80 μ L of dH₂O. After incubation add 100 μ L Stop Reagent.

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Calculations

Subtract blank OD (water, #4) from the standard OD values and plot the ΔOD against standard concentrations. Determine the Slope and use the following equation to calculate α -Fucosidase activity:

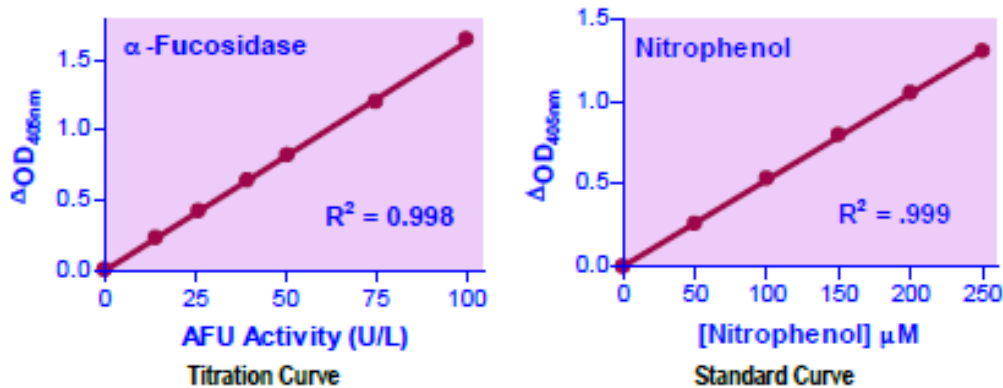
$$\begin{aligned} \text{AFU Activity} &= \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{\text{Time} \cdot \text{Slope}} \times \frac{\text{Reaction Vol } (\mu\text{L})}{\text{Sample Vol } (\mu\text{L})} \times n \\ &= \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{\text{Slope}} \times \frac{1}{4} \times n \quad (\text{U/L}) \end{aligned}$$

where OD_{SAMPLE} is the $OD_{405\text{nm}}$ value for each sample and OD_{BLANK} is the $OD_{405\text{nm}}$ value of the water (standard #4) or the sample blank if one was used. Slope is the slope of the linear regression fit of the standard points and Time is the reaction time (20 min). Reaction Vol and Sample Vol are 100 μL and 20 μL , respectively. n is the dilution factor.

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

Note: If sample AFU activity exceeds 100 U/L, either use a shorter reaction time or dilute samples in 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.

Sample Data



Version: V.08.09.2018

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