Technical Bulletin

α-L-Fucosidase Assay Kit

Catalog Number MAK444

Product Description

α-L-Fucosidase (AFU) is an enzyme coded by the FUCA1 gene in humans and which 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.

The non-radioactive, colorimetric α-L-Fucosidase Assay Kit 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. The assay method has a linear detection range of 1 to 100 units/L for a 20 minute reaction using a 10 µL sample.

The kit is suitable for the quantitative determination of α-L-Fucosidase activity in plasma, serum, tissue, cell lysate, etc.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Substrate Buffer 10 mL
  Catalog Number MAK444A
- Stop Reagent 12 mL
  Catalog Number MAK444B
- Standard (12.5 mM Nitrophenol) 1 mL
  Catalog Number MAK444C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥ 10,000 × *g*
- Phosphate Buffered Saline (PBS) (Catalog Number P3813 or equivalent)
- Potassium phosphate monobasic (Catalog Number P0662 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate Substrate Buffer to desired reaction temperature (25 °C or 37 °C) prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum and plasma

Serum and plasma samples can be assayed directly.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline, pH 7.4, to remove blood.
2. Homogenize tissue (50 mg) in 200 µL of buffer containing 50 mM potassium phosphate, pH 7.5.
3. Centrifuge homogenized tissue at 10,000 × *g* for 15 minutes at 4 °C.
4. Remove supernatant and retain for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 x *g* for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate, pH 7.5.
4. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
5. Remove supernatant and retain for assay.

All Samples

1. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
2. All samples can be stored at -20 °C to -80 °C for at least one month.
3. Transfer 20 µL of each Sample to separate wells of a clear 96-well plate.
4. For visually colored or opaque samples, prepare a Sample Blank by transferring 20 µL of the Sample into parallel wells of the plate. Add 80 µL of purified water to the Sample Blank well.

Standard Curve Preparation

1. Prepare a 250 µM Nitrophenol Standard by mixing 10 µL of the 12.5 mM Nitrophenol Standard with 490 µL of purified water.
2. Prepare Nitrophenol Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Nitrophenol Standards

Well	250 µM Nitrophenol Standard	Purified Water	Nitrophenol (µM)
1	200 µL	-	250
2	120 µL	80 µL	150
3	60 µL	140 µL	75
4	-	200 µL	0

3. Mix well and transfer 100 µL of each Standard into separate wells of a clear 96-well plate.

Measurement

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate Buffer and Stop Reagent to samples should be quick and mixing should be brief but thorough. Use of a multichannel pipettor is recommended.

1. Add 80 µL of Substrate Buffer to each Sample well. Tap plate briefly to mix.
2. Incubate at 25 °C or desired temperature for 20 minutes.
3. Add 100 µL of Stop Reagent to each Sample, Standard, and Sample Blank well. Tap plate briefly to mix.
4. Read optical density (OD) at 405 nm.

Results

1. Subtract the OD_{Blank} (Standard #4) reading from the remaining Standard OD readings. Plot the corrected Standard OD readings against the Standard concentrations.
2. Determine the slope of the Standard curve using linear regression.
3. Calculate the α-L-Fucosidase concentration of the sample:

α-L-Fucosidase (U/L) =

$$\frac{OD_{Sample} - OD_{Blank}}{Time \times Slope} \times \frac{RV\ (\mu L)}{SV\ (\mu L)} \times DF$$

=

$$\frac{OD_{Sample} - OD_{Blank}}{Slope} \times \frac{1}{4} \times DF$$

where

OD_{Sample} = OD value at A_{405} nm of Sample

OD_{Blank} = OD value at A_{405} nm of Blank (Standard #4)

Time = Reaction time (20 minutes)

Slope = Slope of the Standard curve

RV (µL) = Total reaction volume (100 µL)

SV (µL) = Sample volume used in reaction (20 µL)

DF = Sample Dilution Factor (DF = 1 for undiluted Samples)

If the sample AFU activity exceeds 100 U/L, repeat the assay and either use a shorter reaction time or dilute samples in purified water. For samples with AFU activity <5 U/L, the incubation time can be extended up to 40 minutes for greater sensitivity.

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

Figure 1.
Typical Nitrophenol Standard Curve

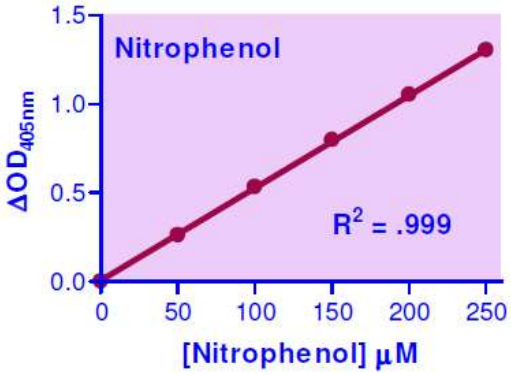
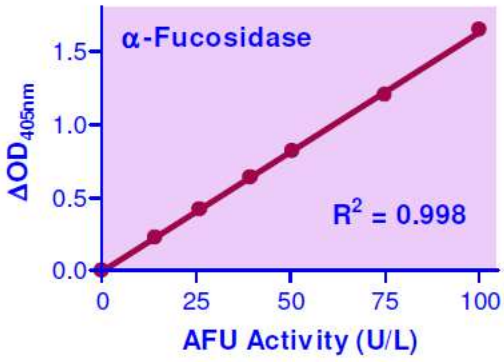


Figure 2.
Typical Titration Curve



References

1. Giardina, M.G., et al., Serum α-L-fucosidase. A useful marker in the diagnosis of hepatocellular carcinoma. *Cancer,* **70,** 1044-1048 (1992)
2. Fernandez-Rodriguez, A.D., et al., Value of the serum α-L-fucosidase activity in the diagnosis of colorectal cancer. *Oncology* **59(4)**, 310-316 (2000).
3. Alhadeff, J.A., et al., Human liver α-L-fucosidase. Purification, characterization, and immunochemical studies. *J. Biol. Chem.,* **250,** 7106-7113 (1975).

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