PRODUCT INFORMATION & MANUAL

alpha-L-Fucosidase Activity Assay Kit (Colorimetric)

NBP3-24550

For research use only.
Not for diagnostic or therapeutic procedures.

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αlphα-L-Fucosidαse Activity Assαy Kit (Colorimetric)

Catalog No: NBP3-24550

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.5 U/L

Detection range: 0.5 - 80 U/L

Average intra-assay CV (%): 3.7

Average inter-assay CV (%): 8.4

Average recovery rate (%): 97

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure α-L-Fucosidase (AFU) activity in serum (plasma) and animal tissue samples.

▲Detection principle

α-L-fucosidase (AFU) is an acid hydrolase that exists in organisms and widely exists in tissues, cells, blood and body fluids. AFU is involved in the metabolism of glycoproteins, glycolipids and oligosaccharides, and plays a key role in cell differentiation, apoptosis, inflammation and host-pathogen interaction. AFU catalyzes the hydrolysis of the colorless substrate nitrophenyl-α-L-fucoside to produce yellow nitrophenol with a significant absorption peak at 405 nm, and its absorbance is positively correlated with the content of nitrophenol. The activity of AFU can be calculated by measuring the change of absorbance value at 405 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Working Solution	25 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 3	5 mmol/L Standard Solution	1 mL × 2 vials	2-8℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Incubator, centrifuge, Microplate reader (400-420 nm, optimum wavelength: 405 nm)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. It is recommended to use clarified serum samples. Hemolysis, hyperlipemia, chylous samples will affect the results.
2. It is recommended to use fresh sample for detection.

Pre-assay preparation

▲ Reagent preparation

Bring reagent 2 and reagent 3 to room temperature before use, and place the reagent 1 on the ice box.

▲ Sample preparation

1. Serum and plasma samples:

Detect the sample directly.

2. Tissue sample:

Accurately weigh the tissue, add reagent 1 at a ratio of weight (g): volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant. If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.5 - 80 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	1
Rat plasma	1
10% Rat liver tissue homogenate	1-3
10% Mouse liver tissue homogenate	1-3
10% Rat spleen tissue homogenate	1
10% Mouse kidney tissue homogenate	1

Note: The diluent is reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 5 mmol/L standard solution with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	5 mmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	100
B	0.5	10	90
C	1.0	20	80
D	1.5	30	70
E	2.0	40	60
F	3.0	60	40
G	4.0	80	20
H	5.0	100	0

2. The measurement of samples

(1) Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

(2) Add 200 μL of regent 2 to each well.

(3) Mix fully with microplate reader and incubate at 37℃ for 15 min. Measure the OD value of each well at 405 nm with microplate reader, recorded as A_1.

(4) Incubate at 37℃ for 30 min. Measure the OD value of each well at 405 nm with microplate reader, recorded as A_2.

Plot the standard curve with the OD value of A_2, calculate the change OD value of sample, $\Delta A = A_2 - A_1$.

▲Summary operation table

	Standard well	Sample well
Standard solution with different concentrations (μL)	20	
Sample (μL)		20
Regent 2 (μL)	200	200
Mix fully and incubate at 37℃ for 15 min. Measure the OD value of each well, recorded as A_1.		
Incubate at 37℃ for 30 min. Measure the OD value of each well, recorded as A_2.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum/plasma :

Definition: The amount of AFU in 1 L liquid sample per 1 minute that hydrolyze the substrate to produce 1 μmol 2-chloro-4-nitrophenol at 37°C is defined as 1 unit.

$$\text{AFU activity (U/L)} = (\Delta A - b) \div a \div T \times 1000^{*} \times f$$

2. Tissue:

Definition: The amount of AFU in 1 g tissue protein per 1 minute that hydrolyze the substrate to produce 1 μmol 2-chloro-4-nitrophenol at 37°C is defined as 1 unit.

$$\text{AFU activity (U/gprot)} = (\Delta A - b) \div a \div T \times 1000^{*} \div C_{pr} \times f$$

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA: The change OD values of sample well, A_2-A_1.

T: The time of reaction, 30 min..

1000*: 1 mmol = 1000 μmol

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

Appendix I Data

▲Example analysis

For human serum, take 20 μL human serum, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.2492 x – 0.0023, the A_1 of the sample is 0.213, the A_2 of the sample is 0.338, $\Delta A = 0.338 - 0.213 = 0.125$, and the calculation result is:

AFU activity (U/L) = (0.125 + 0.0023) ÷ 0.2492 ÷ 30 × 1000 = 17.03 U/L