（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K762-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（400-420 nm）

Detection range：0.5 - 80 U/L

Elabscience®α-L-Fucosidase(AFU) Acitivity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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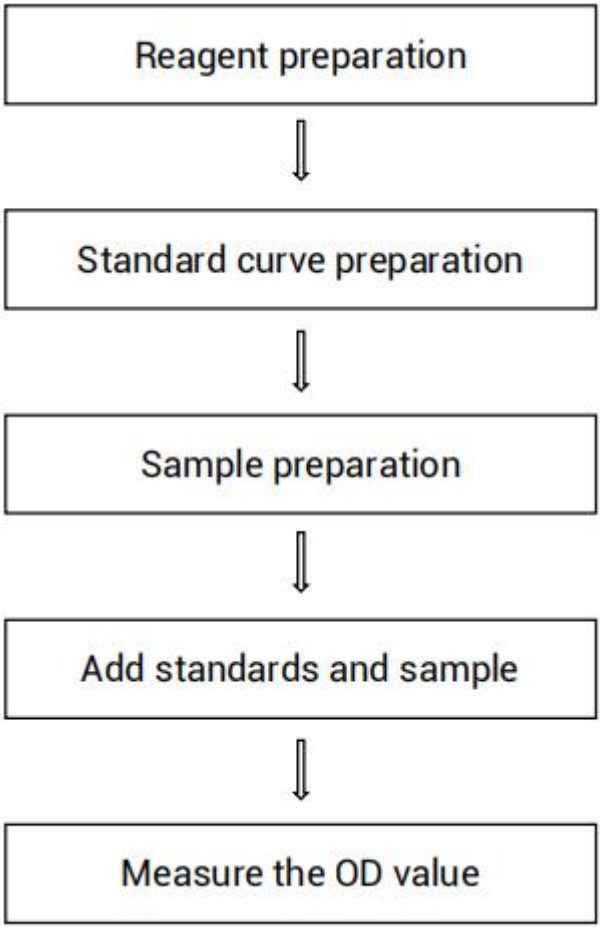
Assay summary



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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	2-8℃, 12 months
Reagent 2	Working Solution	12 mL × 1 vial	25 mL × 1 vial	2-8 ℃, 12 months, shading light
Reagent 3	5 mmol/L Standard Solution	1 mL × 1 vial	1 mL × 2 vials	2-8 ℃, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Keep extraction solution on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 5 mmol/L standard solution with extraction solution 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.5**	**1.0**	**1.5**	**2.0**	**3.0**	**4.0**	**5.0**
5 mmol/L standard (μL)	0	10	20	30	40	60	80	100
Extraction solution (μL)	100	90	80	70	60	40	20	0

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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 extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

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.

Operating steps

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 working solution 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

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.

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

2. Tissue sample:

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

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

[Note]

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

T: The time of reaction, 30 min.

1000*: 1 mmol = 1000 μmol.

Cpr: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.50	34.60	72.00
%CV	4.0	3.6	3.5

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	3.50	34.60	72.00
%CV	8.2	8.5	8.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.7	1.8	3.3
Observed Conc. (mmol/L)	0.7	1.7	3.2
Recovery rate (%)	97	96	98

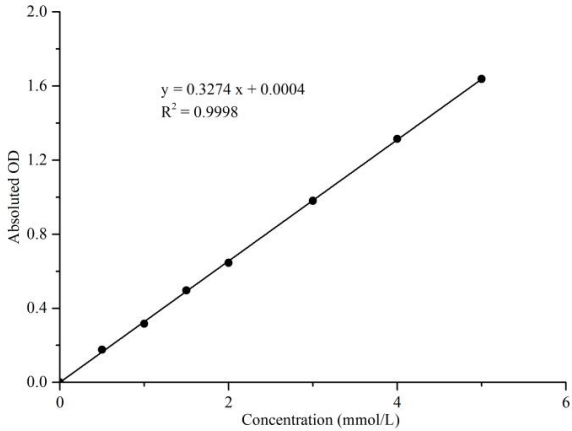
Sensitivity

The analytical sensitivity of the assay is 0.5 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	0	0.5	1	1.5	2	3	4	5
Average OD	0.093	0.269	0.410	0.590	0.739	1.073	1.407	1.731
Absoluted OD	0.000	0.176	0.317	0.497	0.646	0.980	1.314	1.638



Appendix Π Example Analysis

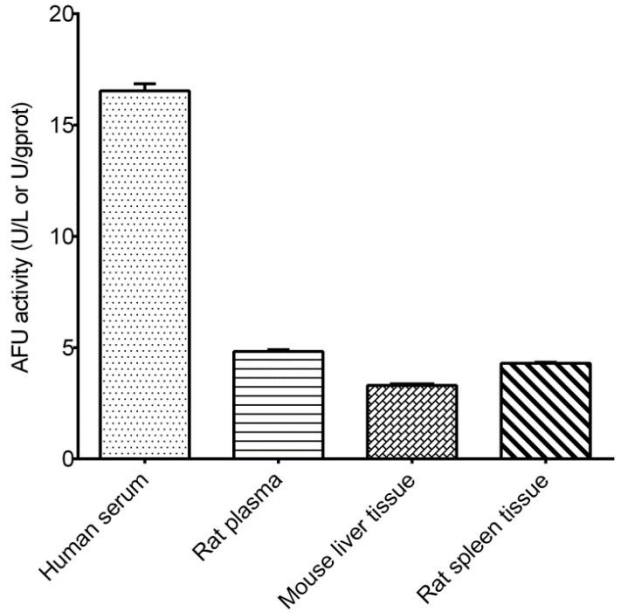
Example analysis：

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 A1 of the sample is 0.213, the A2 of the sample is 0.338, ΔA = 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

Detect human serum, rat plasma, 10% mouse liver tissue homogenate (the concentration of protein is 7.47 gprot/L) and 10% rat spleen tissue homogenate (the concentration of protein is 3.32 gprot/L) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.