alpha-L-Fucosidase Microplate Assay Kit

Catalog # AS0158

Detection and Quantification of alpha-L-Fucosidase Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION

alpha-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 caricinoma. 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.

alpha-L-Fucosidase Microplate 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.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	-20 °C
Reaction Buffer	10 ml x 1	4 °C
Stop Solution	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 8 ml Reaction Buffer to dissolve before use.

Standard:add 1 ml Reaction Buffer to dissolve before use, then add 30 µl into 970 µlReaction Buffer, mix; the concentration will be 300 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquidsamples

Detect directly.

V. ASSAY PROCEDURE

Warm all regentsto room temperature before use.

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Substrate	80 µl	--	--
Mix, put it in the oven, 37°C for 30 minutes.			
Stop Solution	100 µl	100 µl	100 µl
Mix, record absorbance measured at 405 nm.			

VI. CALCULATION

Unit Definition: One unit ofAFU activity is defined as the enzyme generates 1μmol of p-nitrophenol per minute.

1. According to the protein concentration of sample

$$AFU\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$AFU\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3.According to the quantity of cells or bacteria

$$AFU\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 300μmol/L = 0.3μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N ×10^4;

$V_{Standard}$: the volume of standard, 0.1 ml;

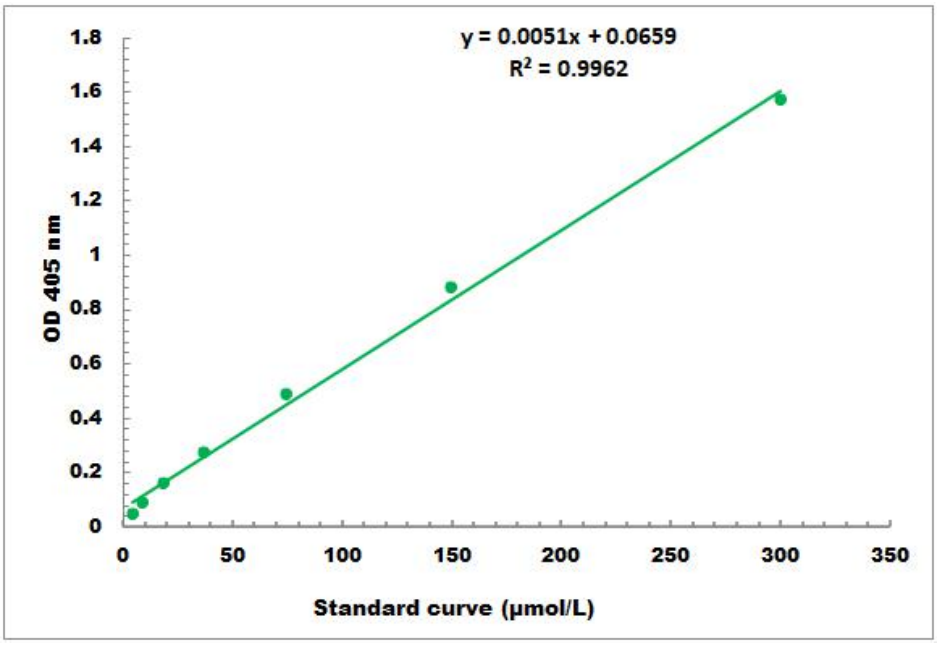
V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 30 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 3μmol/L -300μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES