α-L-Fucosidase (AFU) Activity Assay Kit

Cat. No. KITG-088 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	α-L-Fucosidase (α-L-Fucosidase, AFU) is a lysosomal acidic hydrolase widely present in human tissue cells, blood, and body fluids. It is mainly involved in the catabolism of glycoproteins, glycolipids, and oligosaccharides. Serum AFU activity is used as an important biological marker for primary liver cancer and also shows abnormal activity in liver cirrhosis, chronic hepatitis, and other diseases. α-L-Fucosidase hydrolyzes p-nitrophenyl-α-L-fucopyranoside to produce α-L-fucose and p-nitrophenol. p-Nitrophenol has a characteristic absorption peak at 405 nm. The activity of α-L-fucosidase can be determined by measuring the change in absorbance at 405 nm.
Description	Method: Visible spectrophotometry. Detection wavelength: 405 nm. Assay principle: α-L-Fucosidase hydrolyzes p-nitrophenyl-α-L-fucopyranoside to generate α-L-fucose and p-nitrophenol. The p-nitrophenol product has a characteristic absorption peak at 405 nm, and the change in absorbance is used to determine α-L-fucosidase activity. Size: 50T/24S. Storage temperature: 2-8°C.
Form	Kit
Applications	Determination of α-L-fucosidase activity in tissue samples, bacteria or cells, serum or plasma, culture medium, and other liquid samples.
Notes	Required Instruments and Reagents Visible spectrophotometer; 1 mL glass cuvette with a 10 mm optical path, 3 mm slit, and 1.05 mL volume; mortar/homogenizer; adjustable pipette; benchtop centrifuge; constant-temperature water bath/incubator;

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	and distilled water. Preparation of Crude Enzyme Extract (the sample amount and ratio may be adjusted appropriately according to preliminary experimental results) 1. Tissue: Treat the sample at a tissue mass (g): Extraction Solution volume (mL) ratio of 1:(5-10). It is recommended to weigh 0.1 g tissue and add 1 mL Extraction Solution. Homogenize in an ice bath, centrifuge at 10,000 × g for 10 min at 4°C, and keep the supernatant on ice for testing. 2. Bacteria or cells: Collect bacteria or cells by centrifugation. Treat the sample at a bacteria or cell number (10^4): Extraction Solution volume (mL) ratio of (500-1000):1. It is recommended to add 1 mL Extraction Solution to 5 × 10^6 bacteria or cells. Disrupt the bacteria or cells by sonication in an ice bath at 200 W, with 3 s sonication and 7 s intervals, for a total time of 3 min. Centrifuge at 10,000 × g for 10 min at 4°C, and keep the supernatant on ice for testing. 3. Serum (plasma), culture medium, and other liquid samples: Test directly or after appropriate dilution. If a liquid sample is turbid, centrifuge at 10,000 × g for 10 min at 4°C and keep the supernatant on ice for testing. Precautions 1. If ΔA_sample is greater than 1.0, appropriately dilute the crude enzyme extract with distilled water before measurement. If ΔA_sample is less than 0.05, appropriately extend the enzymatic reaction time. The reaction is normally performed accurately at 37°C for 30 min and may be extended to 60 min or longer, or a more concentrated sample may be prepared before measurement. Modify the calculation accordingly. 2. Keep the crude enzyme extract on ice before measurement. It is recommended to complete the activity assay on the same day as extraction. 3. To ensure accurate results and avoid reagent loss, carefully read the instructions before measurement, confirm that reagent storage and preparation are adequate and that the operating steps are clear, and perform a preliminary assay using 2-3 samples expected to show substantial differences.
Storage	Store the kit at 2-8°C. Extraction Solution: Liquid, 60 mL × 1 bottle; store at 4°C. Reagent 1: Liquid, 12 mL × 1 bottle; store at 4°C. Reagent 2: Liquid, 1 mL × 1 vial; store at 4°C. Reagent 3: Liquid, 50 mL × 1 bottle; store at 4°C. If crystals precipitate,

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	dissolve them by ultrasonic treatment at 37°C before use. Standard Solution: Liquid, 1 mL × 1 vial; store at 4°C. The solution is a 5 μmol/mL p-nitrophenol standard solution.
Reconstitution	Standard Application Solution Preparation (prepare fresh before use): Dilute the 5 μmol/mL p-nitrophenol standard solution with distilled water to 100 nmol/mL. This is the standard application solution. Reagent 2 Application Solution Preparation (prepare fresh before use): Before use, prepare the solution according to the required amount by mixing Reagent 1 and Reagent 2 at a volume ratio of 6:1.
Reaction conditions	Assay Procedure 1. Preheat the spectrophotometer for more than 30 min, set the wavelength to 405 nm, and zero with distilled water. 2. Prepare the Reagent 2 application solution immediately before use by mixing Reagent 1 and Reagent 2 at a volume ratio of 6:1. 3. Add the following reagents sequentially to centrifuge tubes: Sample Tube: Add 200 μL crude enzyme extract and 200 μL Reagent 2 application solution. Control Tube: Add 200 μL crude enzyme extract and 200 μL Reagent 1. Mix thoroughly and react accurately at 37°C for 30 min. Standard Tube: Add 400 μL standard application solution. Blank Tube: Add 400 μL distilled water. Add 720 μL Reagent 3 to the sample, control, standard, and blank tubes, and mix thoroughly. Absorbance Measurement Transfer 1 mL of each reaction mixture to a 1 mL glass cuvette and measure the absorbance at 405 nm. Record the values as A_sample, A_control, A_standard, and A_blank. Calculate ΔA_sample = A_sample - A_control and ΔA_standard = A_standard - A_blank. A separate control tube is required for each sample. The standard and blank tubes only need to be measured 1-2 times.
Unit definition	α-L-Fucosidase (AFU) Activity Calculation 1. Calculation Based on Tissue Protein Concentration Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol p-nitrophenol per hour per mg tissue protein. AFU (U/mg prot) = [C_standard × ΔA_sample × V_reaction × D] / [ΔA_standard × V_sample × Cpr × T] = [400 × ΔA_sample × D] / [ΔA_standard × Cpr]. 2. Calculation

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	Based on Tissue Sample Mass Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol p-nitrophenol per hour per g tissue. AFU (U/g) = [C_standard × ΔA_sample × V_reaction × V_extraction × D] / [ΔA_standard × V_sample × W × T] = [400 × ΔA_sample × D] / [ΔA_standard × W]. 3. Calculation Based on Bacteria or Cell Number Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol p-nitrophenol per hour per 10^4 bacteria or cells. AFU (U/10^4 cells) = [C_standard × ΔA_sample × V_reaction × V_extraction × D] / [ΔA_standard × V_sample × bacteria or cell number × T] = [400 × ΔA_sample × D] / [ΔA_standard × bacteria or cell number]. 4. Calculation Based on Liquid Sample Volume Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol p-nitrophenol per hour per mL liquid sample. AFU (U/mL) = [C_standard × ΔA_sample × V_reaction × D] / [ΔA_standard × V_sample × T] = [400 × ΔA_sample × D] / ΔA_standard. Where: C_standard is the concentration of the standard application solution, 100 nmol/mL; V_reaction is the total volume of the enzymatic reaction system, 0.4 mL; V_sample is the volume of crude enzyme extract added to the reaction system, 0.2 mL; V_extraction is the volume of Extraction Solution added during extraction, 1 mL; Cpr is the protein concentration of the crude enzyme extract, mg/mL; bacteria or cell number is expressed in units of 10^4, so 5 × 10^6 bacteria or cells should be entered as 500; W is the sample mass, g; T is the enzymatic reaction time, 0.5 h; D is the dilution factor of the crude enzyme extract, and is 1 when the extract is not diluted.
Usage	For Research Use Only. Not for Use in Diagnostic Procedures.

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