Alcohol Acyl Transferase(AAT) Activity Assay Kit

Product Code: 112885

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Method: Microplate method

Product Introduction

Alcohol acyl transferase (AAT) belongs to a large family of multifunctional proteins that catalyze acylation and deacylation reactions in organisms. AAT plays important roles in gene expression, metabolism, and signal transduction.

AAT catalyzes the transfer of acetyl groups from acetyl-CoA to butanol. The released CoA reduces DTNB to generate TNB. TNB has an absorption peak at 412 nm, and the increase in absorbance at 412 nm can be used to calculate AAT activity.

Package Contents

Code	Component	Quantity
112885.1	Reagent I	1 bottle
112885.2	Reagent II	1 bottle
112885.3	Reagent III	1 bottle
112885.4	Extraction Solution	1 bottle
112885.m	Instruction Manual	1 copy

Quality and Safety Information

Material	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Reagent III	--	--
Extraction Solution	--	--

Transportation and Storage

Shipping	Shipped with ice packs.
Storage	Store Reagent II at -20°C. Store other components at 2-8°C protected from light. Shelf life: 180 days.

Instructions for Use

1. Preparation of Crude Enzyme Extract

1. **Tissue:** Use a tissue mass (g) to Extraction Solution volume (mL) ratio of 1:5-10. It is recommended to weigh approximately 0.1 g tissue and add 1 mL Extraction Solution. Homogenize in an ice bath, then centrifuge at 8000g for 10 min at 4°C. Collect the supernatant and keep it on ice for testing.

2. **Bacteria and fungi:** Use a cell number (10^4cells) to Extraction Solution volume (mL) ratio of 500-1000:1. It is recommended to add 1 mL Extraction Solution to 5,000,000 cells. Disrupt cells by ultrasonic treatment in an ice bath at 300 W, with ultrasound for 3 seconds and an interval of 7 seconds, for a total time of 3 min. Centrifuge at 8000g for 10 min at 4°C. Collect the

supernatant and keep it on ice for testing.

3. **Liquid samples:** Test directly.

2. Assay Procedure

1. Preheat the microplate reader for 30 min. Set the wavelength to 412 nm and zero with distilled water.
2. Prepare the working solution before use by adding 18 mL Reagent I to the Reagent II bottle. Mix thoroughly and set aside. Aliquot unused reagent and store at -20°C. Repeated freezing and thawing is prohibited.
3. Prepare Reagent III immediately before use by adding 1 mL anhydrous ethanol. Dissolve completely and set aside. Store unused reagent at 4°C.
4. Blank well: add 10 μL extract and 180 μL working solution to a 96-well plate. Mix thoroughly and react at 35°C for 15 min. Add 10 μL Reagent III, mix thoroughly, and let stand at 25°C for 10 min. Measure absorbance at 412 nm and record as A_{blank}.
5. Assay well: add 10 μL sample and 180 μL working solution to a 96-well plate. Mix thoroughly and react at 35°C for 15 min. Add 10 μL Reagent III, mix thoroughly, and let stand at 25°C for 10 min. Measure absorbance at 412 nm and record as A_{assay}.

Calculate ΔA as follows: $\Delta A = A_{assay} - A_{blank}$. Only one blank well is required.

3. Calculation

Use the following formulas for the 96-well plate assay.

Calculation According to Protein Concentration

Definition of activity unit: the amount of enzyme that catalyzes the production of 1 nmol TNB per milligram of protein per minute is defined as one enzyme activity unit.

$$AAT\ (nmol/min/mg\ prot) = \Delta A \div (\varepsilon \times d) \times V_{total\ reaction} \div (V_{sample} \times Cpr) \div T = 196.08 \times \Delta A \div Cpr$$

Calculation According to Sample Mass

Definition of activity unit: the amount of enzyme that catalyzes the production of 1 nmol TNB per gram of tissue per minute is defined as one enzyme activity unit.

$$AAT\ (nmol/min/g\ fresh\ weight) = \Delta A \div (\varepsilon \times d) \times V_{total\ reaction} \div (W \times V_{sample} \div V_{total\ sample}) \div T = 196.08 \times \Delta A \div W$$

Calculation According to Cell Number

Definition of activity unit: the amount of enzyme that catalyzes the production of 1 nmol TNB per 10^4cells per minute is defined as one enzyme activity unit.

$$AAT\ (nmol/min/10^4 cells) = \Delta A \div (\varepsilon \times d) \times V_{total\ reaction} \div (cell\ number \times V_{sample} \div V_{total\ sample}) \div T = 196.08 \times \Delta A \div cell\ count$$

Calculation According to Liquid Volume

Definition of activity unit: the amount of enzyme that catalyzes the production of 1 nmol TNB per milliliter of sample per minute is defined as one enzyme activity unit.

$$AAT\ (nmol/min/mL) = \Delta A \div (\varepsilon \times d) \times V_{total\ reaction} \div V_{sample} \div T = 196.08 \times \Delta A$$

Formula Parameters

ε	TNB extinction coefficient: 13600 L/mol/cm
d	96-well plate optical path length: 0.5 cm
$V_{total\ reaction}$	Total reaction volume: 0.2 mL
V_{sample}	Volume of supernatant added to the reaction system: 0.01 mL
$V_{total\ sample}$	Volume of Extraction Solution added: 1 mL
Cpr	Protein content: mg/mL

W	Sample mass: g
T	Reaction time: 15 min

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for a preliminary test.
2. Required instruments and supplies not provided: mortar, ice, benchtop centrifuge, microplate reader, 96-well plate, constant-temperature water bath, and absolute ethanol.
3. This 100T kit tests 96 samples.