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CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit

Cat #: KTB1560 Size: 48 T/96 T

	Micro Alcohol Acyltransferase (AAT) Activity Assay Kit		
REF	**Cat #:** KTB1560	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria,		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

AAT is a large family of multifunctional proteins, mainly responsible for catalyzing various acylation and deacylation reactions in vivo, and plays an important role in gene expression, metabolism and signal transduction. CheKine™ Micro Alcohol Acyltransferase (AAT) Activity Assay Kit provides a convenient tool for detection of AAT activity. The principle is that AAT catalyzes the transfer of acetyl group from acetyl CoA to butanol, and at the same time reduces DTNB to TNB. TNB has an absorption peak at 412 nm. The enzyme activity of AAT was calculated by detecting the rate of increase in absorption at 412 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Receptor	10 mL	20 mL	4℃
Substrate	1	1	-20℃
Chromogen	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, ice maker, refrigerated centrifuge
- Anhydrous ethanol
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Receptor: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Substrate: Add 18 mL Receptor for 96 T or 9 mL Receptor for 48T to dissolve before use. This solution can be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.

Chromogen: Add 1 mL anhydrous ethanol for 96 T or 0.5 mL anhydrous ethanol for 48 T to dissolve before use. Stored at 4℃.

Working Reagent: Prepare 190 μL Work Reagent for one well, add 180 μL of dissolved Substrate and 10 μL Chromogen. Prepare Working Reagent before use and depend on your need.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80 ℃ for one month.Processed samples must be assayed immediately.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Cells or bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
4. Serum, plasma, or other liquid samples: Tested directly.

Note: For animal tissues with high fat content, remove the upper layer of fat after centrifugation, and then take the supernatant. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm, visible spectrophotometer was returned to zero with deionized water.
2. Preheat the incubator to 37℃. Working Reagent is placed in incubator to preheat for 15 min.
3. Add 10 μL of sample or Extraction Buffer, 190 μL of Working Reagent to the 96-well plate or microglass cuvette, then tap the plate and mix well, quickly. Incubate at 37℃ for 2 min, then measure absorbance value at 412 nm. The sample absorbance value is recorded as A_{Test} and Extraction Buffer absorbance value is recorded as A_{Blank}, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.5, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well plates calculation formula

1. Calculated by protein concentration

Unit definition: 1 nmol TNB produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

$AAT\ (U/mg\ prot)=(\Delta A_{Test}\div\varepsilon\div d\times V_{Reaction\ Total}\times10^9)\div(Cpr\times V_{sample})\div T\times n$**$=1,470.6\times\Delta A_{Test}\div Cpr\times n$**

2. Calculated by fresh weight of samples

Unit definition: 1 nmol TNB produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

AAT (U/g)=(ΔA_{Test}÷ε÷d×$V_{Reaction\ Total}$×10^9)÷(W×V_{Sample}÷$V_{Sample\ Total}$)÷T×n=**1,470.6×ΔA_{Test}÷W×n**

3. Calculated the activity of AAT by cells or bacteria number

Unit definition: 1 nmol TNB produced per min in 10^4 cells or bacteria reaction system is defined as a unit of enzyme activity.

AAT ($U/10^4$)=(ΔA_{Test}÷ε÷d×$V_{Reaction\ Total}$×10^9)÷(total number×V_{Sample}÷$V_{Sample\ Total}$)÷T×n=1,470.6×ΔA_{Test}÷500×n=**2.9412×ΔA_{Test}×n**

4. Calculate the activity of AAT in liquid sample

Unit definition: 1 nmol TNB produced per min in 1mL liquid sample reaction system is defined as a unit of enzyme activity.

AAT (U/mL)=(ΔA_{Test}÷ε÷d×$V_{Reaction\ Total}$×10^9)÷V_{Sample}÷T×n=**1,470.6×ΔA_{Test}×n**

Where: ε: TNB molar extinction coefficient, 13.6×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; $V_{Reaction\ Total}$: total reaction volume, 200 μL=2×10^{-4} L; 10^9: 1 mol=1×10^9 nmol; Cpr: sample protein concentration, mg/mL; V_{sample}: sample volume added, 0.01 mL; T: reaction time, 2 min; n: dilution factor; W: sample weight, g; $V_{Sample\ Total}$: Extraction Buffer volume added, 1 mL; 500: total number of cells or bacteria, 5×10^6.

B. Microglass cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

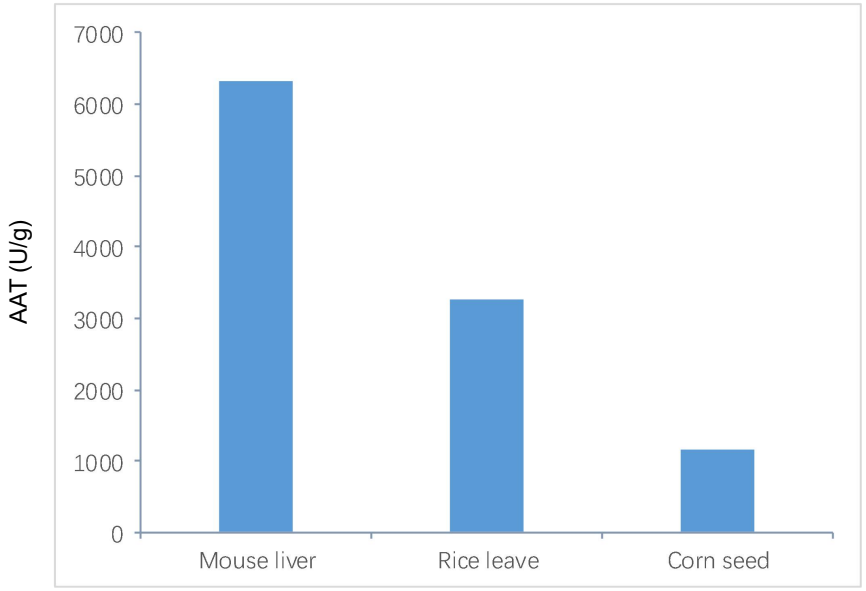

Figure 1. AAT activity in mouse liver, rice leave and corn seed respectively. Assays were performed following kit protocol.

Recommended Products

Catalog No.	Product Name
KTB1125	CheKine™ Micro Pyruvate Decarboxylase (PDC) Assay Kit
KTB2230	CheKine™ Micro Free Fat Acid (FFA) Assay Kit
KTB2240	CheKine™ Micro Fatty Acid Synthetase (FAS) Activity Assay Kit

Disclaime

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.