Acyltransferase (AAT) Activity Assay Kit

Note: The reagents have changed, please operate in strict accordance with the instructions.

Operation Equipment: Spectrophotometer

Catalog Number: BC2350

Size: 50T/48S

Components:

Extraction Reagent: 60 mL×1. Storage at 2-8℃. The reagent contains insoluble matter, shake well before use.

Reagent I: Liquid 50 mL×1. Storage at 2-8℃.

Reagent II: Powder×2. Storage at -20℃. Before use, add 1.5 mL of distilled water to fully dissolve it. Unused reagents can be stored for 2 weeks at 2-8℃.

Reagent III: Liquid 6 mL×1. Storage at 2-8℃.

Reagent IV: Liquid 3 mL×1. Storage at 2-8℃.

Product Description

Acyltransferases are a large family of multifunctional proteins, which are mainly responsible for catalyzing various acylation and deacylation reactions in the body, playing an important role in gene expression, metabolism and signaling.

Acyltransferase catalyzes acetyl CoA to transfer acetyl to butanol, and at the same time reduces DTNB to generate TNB; TNB has an absorption peak at 412 nm, and the rate of increase in absorbance at 412 nm is measured to calculate Acyltransferase activity.

Reagents and Equipment Required but Not Provided.

Spectrophotometer, centrifuge, water bath / constant temperature incubator, 1 mL glass cuvette, adjustable pipette, mortar/homogenizer, ice and distilled water

Sample pre-treatment:

1) Tissue sample:

According to the ratio of tissue mass (g): extraction reagent volume (mL) to 1:5~10 (it is recommended to weigh about 0.1g of tissue, add 1mL of extraction reagent), and perform ice bath homogenization. 4℃, 15000g centrifugation for 20min, take the supernatant for testing.

2) Serum sample:

Detect sample directly. (If the liquid is turbid, measure after centrifugation)

Procedure and Sample list

1. Preheat the spectrophotometer for more than 30 min, adjust the wavelength to 412 nm, and set zero with distilled water.

2. Reagent 1 is incubated in a water bath at 37°C for more than 20 minutes.
3. Operation

Reagent name (mL)	Blank Tube (A_B)	Test Tube (A_T)

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Distilled water	0.1	-
Sample	-	0.1
Reagent I	0.7	0.7
Reagent II	0.05	0.05
Reagent III	0.1	0.1
Reagent IV	0.05	0.05
Add the above reagents to a 1 mL glass cuvette in order, start counting while adding Reagent IV, record the initial absorbance A1 at 10s at 412 nm and absorbance A2 after 130s, and calculate $\Delta A_B = A_B2-A_B1$; $\Delta A_T = A_T2 - A_T1$, $\Delta A = \Delta A_T - \Delta A_B$.		

Calculation

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the absorbance changing of 0.001 in 1 mL reaction system per minute at 37℃ every milligram protein.

Acyltransferases activity (U/mg prot) =ΔA÷0.001 ÷ (Vs× Cpr) ÷ T=5000×ΔA÷Cpr.

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the absorbance changing of 0.001 in 1 mL reaction system per minute at 37℃ every gram sample.

Acyltransferases activity (U/g weight) =ΔA÷0.001 ÷ (Vs÷ Ve×W) ÷ T=5000×ΔA÷W.

3. serum:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the absorbance changing of 0.001 in 1 mL reaction system per minute at 37℃ every milliliter serum.

Acyltransferases activity (U/mL) =ΔA÷0.001 ÷ Vs ÷ T=5000×ΔA.

Cpr: Supernatant protein concentration, mg/mL;

T: Reaction time, 2 min;

Vs: Sample volume, 0.1 mL;

Ve: Extraction reagent volume, 1 mL;

W: Sample weight, g.

Notes:

1. The protein content of supernatant should be determined separately.
2. When the absorbance value is greater than 1, it is recommended to measure after dilution.
3. If ΔA is low, the reaction time can be prolonged, such as the absorbance of 10s and 310s, and the reaction time in the calculation formula can be modified accordingly.

Experimental Example:

1. Take 0.1g kidney and add 1mL extract for sample processing. After the supernatant was diluted 4 times,

the operation is carried out according to the determination steps. Using micro quartz cuvette, the results showed that $\Delta A_B = A2_B-A1_B$ =0.105-0.101=0.004, $\Delta A_T = A2_T-A1_T$ =0.665-0.479=0.186, $\Delta A = \Delta A_T - \Delta A_B$ =0.186-0.004=0.182

AAT (U/g mass) = 5000×ΔA ÷ W×4 (dilution ratio) = 36400 U/g mass.

2. Take the rabbit serum and directly follow the determination steps The results showed that $\Delta A_B = A2_B - A1_B$ =0.105-0.101=0.004, $\Delta A_T = A2_T - A1_T$ =0.622-0.521=0.101, $\Delta A = \Delta A_T - \Delta A_B$ = 0.101-0.004=0.097.

AAT (U/mL serum) = 5000 × ΔA = 5000×0.097=485 U/mL serum.

Related Products:

BC0590/BC0595	Free fatty Acids (FFA) Content Assay Kit
BC1080/BC1085	Alcohol Dehydrogenase(ADH) Activity Assay Kit
BC0320/BC0325	Plant Lipoxygenase (LOX) Activity Assay Kit

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