Acyltransferase(AAT) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: BC2350

Size: 50T/48S

Components:

Extraction Reagent: 50 mL×1. Storage at 4℃.

Reagent I: Liquid 50 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at -20℃. Add 2.6 mL of distilled water to dissolve the powder before use. The dissolved reagent is stored at 4℃.

Reagent III: Liquid 5 mL×1. Storage at 4℃.

Reagent IV: Powder×1. Storage at -20℃ and protect from light. Add 2.6 mL of Reagent I to dissolve the powder before use. The dissolved reagent is stored at 4℃ and protect from light.

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, analytical balance, 1 mL glass cuvette, distilled water, centrifuge, water bath, adjustable pipette, mortar/homogenizer, ice and distilled water

Sample pre-treatment:

Tissue sample: Weigh about 0.1 g of sample and add 1 mL of Extraction reagent, fully grind on ice, centrifuge at 15000 rpm at 4°C for 20 min, and the supernatant is to be tested.

Serum samples: directly detect.

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 30 minutes.
3. Operation

	Blank Tube (A_B)	Test Tube (A_T)
Distilled water (mL)	0.1	0.1

Sample (mL)	-	0.1
Reagent I (mL)	0.7	0.7
Reagent II (mL)	0.05	0.05
Reagent III (mL)	0.1	0.1
Reagent IV (mL)	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 mg protein.

Acyltransferases activity (U/mg prot) =1000×ΔA ÷ (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 g sample.

Acyltransferases activity (U/g weight) =1000×ΔA ÷ (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 mL serum.

Acyltransferases activity (U/mL) =1000×ΔA ÷ 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. 0.1g kidney is added with 1 mL of Extraction Reagent for sample treatment. 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. 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