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Tissue and Blood Alkaline Phosphatase (AKP/ALP) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0402

Size: 50T/24S

Components:

Extraction reagent: Liquid 30 mL×1 bottle, storage at 4℃ .

Reagent 1: Liquid 10 mL×1 bottle, storage at 4℃ and protect from light.

Reagent 2: Liquid 10 mL×1 bottle, storage at 4℃ and protect from light.

Reagent 3: Liquid 30 mL×1 bottle, storage at 4℃ and protect from light. It cannot be used if it turns to blue-green.

Standard: Liquid 1 mL×1 bottle, 10 μmol/mL phenol standard solution, storage at 4℃ . Dilute with distilled water to 2.5 μmol/mL before use.

Product Description:

AKP/ALP is a zinc-containing glycoprotease, which hydrolysis various natural and synthetic phospholipid monoester compounds in alkaline condition. AKP / ALP are widely distributed in human organs, mainly in liver.

In alkaline condition, AKP/ALP catalyzes hydrolysis disodium phenyl phosphate to phenol, and the phenol reacts with 4-Aminoantipyrine and potassium ferricyanide to form red quinone derivative, which can be detect absorbance at 510 nm. AKP/ALP activity can be calculated by measuring the absorbance increase rate at 510 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, desk centrifuge, transferpettor, 1 mL glass cuvette, ice and distilled water.

Procedure:

I. Enzyme preparation:

Add 1 mL Extraction reagent to 0.1 g tissue, grind thoroughly. Centrifuge at 4℃ 10000 rpm for 10 minutes. Take the supernatant on the ice for test. Blood sample can be detected directly. Dilute with Extraction reagent if concentrate is high.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 510 nm, set the counter to zero with distilled water.
2. Add reagents in 1.5 mL cuvette as the following:

Reagent name (μL)	Test tube A3	Control tube A4	Blank tube A2	Standard tube A1
Distilled water			20	
Standard solution				20
Supernatant	20			
Reagent 1	200	200	200	200
Reagent 2	200	200	200	200
Mix thoroughly, stay in 37℃ for 15 minutes.				
Reagent 3	600	600	600	600
Supernatant		20		
Mix thoroughly, detect absorbance at 510 nm, record as A1, A2, A3, A4.				

IIII. AKP/ALP activity calculation:

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol phenol in the reaction system per minute at 37℃ every mg protein.

AKP/ALP (U/mg prot) =[C×(A3-A4)÷(A1-A2)×Vs]÷(Cpr×Vs)÷T=0. 167×(A3-A4)÷(A1-A2)÷Cpr

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 μmol phenol in the reaction system per minute at 37℃ every g sample.

AKP/ALP (U/g weight) =[C×(A3-A4)÷(A1-A2)×Vs]÷(W÷Ve×Vs)÷T=0. 167×(A3-A4)÷(A1-A2)÷W

3) Blood:

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

AKP/ALP (U/mL) =[C×(A3-A4)÷(A1-A2)×Vs]÷Vs÷T=0. 167×(A3-A4)÷(A1-A2)

C: Standard concentration, 2.5 μmol/mL;

Vrv: Total reaction volume, 1020 μL=1.02 mL;

Vs: Supernatant volume, 0.02 mL;

Ve: Extraction volume, 1 mL;

W: Sample weight, g;

T: Reaction time, 15 minutes;

Cpr: Sample protein concentration, mg/mL.

Note:

1. Reagent 1, Reagent 2 and Reagent 3 should be protected from light.
2. Reagent 3 cannot be used if it has changed to blue-green.
3. Mix thoroughly quickly after adding Reagent 3 to avoid incomplete coloration.

Experimental example:

1. Take 0. 1g of mouse pancreas and add 1 mL of Extract solution for homogenate. After taking the supernatant, operate according to the determination steps. Calculate A_T = 0.324, A_C = 0.037, A_B = 0.022, A_S = 0.677. Calculate the enzyme activity according to the sample mass: AKP/ALP enzyme activity (U/g mass) = 0.167 ×(A_T-A_C)÷(A_S-A_B) ÷ W =0. 167×(0.324-0.037)÷(0.677-0.022)÷0. 1=0.732 U/g mass.

2. After taking the rabbit serum, the operation is carried out according to the determination steps, and the enzyme activity is calculated as follows: A_T = 0. 190, A_C = 0.015, A_B = 0.022, A_S = 0.677. According to the blood volume, the enzyme activity is calculated as follows: AKP/ALP enzyme activity (U/mL) = 0. 167×(A_T-A_C) ÷ (A_S-A_B) =0. 167×(0. 190-0.015)÷(0.677-0.022)=0.0446 U/mL.

Recend Product Citations：

[1] Yang J, Zhang K, Que K, et al. Surface modification of titanium with hydroxyapatite layer induced by phase-transited lysozyme coating[J]. Materials Science and Engineering: C, 2018, 92: 206-215.

[2] Yu Jiang,Dantian Zhu,Wenfeng Liu,et al. Hedgehog pathway inhibition causes primary follicle atresia and decreases female germline stem cell proliferation capacity or stemness. Stem Cell Research & Therapy. July 2019;(IF4.627)

[3] Zhongshi Xu,Feng Dai,Ji Chen,et al. Experimental research into the potential therapeutic effect of GYY4137 on Ovariectomy-induced osteoporosis. Cellular & Molecular Biology Letters. October 2018;(IF3.367)

[4] Wanxiu Cao,Jing Li,Yaoxian Chin,et al. Transcriptomic analysis reveals effects of fucoxanthin on intestinal glucose transport. Journal of Functional Foods. October 2018;(IF3. 197)

Related Products：

AK0410/AK0409	Acetylcholinesterase(AchE) Activity Assay Kit
AK0404/AK0403	Acid Phosphatase(ACP) Activity Assay Kit
AK0514/AK0513	Carboxylesterase(CarE) Activity Assay Kit