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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/microplate reader

Catalog Number: AK0401

Size: 100T/48S

Components:

Extraction reagent: Liquid 60 mL×1 bottle, storage at 4℃ .

Reagent 1: Liquid 5 mL×1 bottle, storage at 4℃ and protect from light.

Reagent 2: Liquid 5 mL×1 bottle, storage at 4℃ and protect from light.

Reagent 3: Liquid 15 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/ microplate reader, micro glass cuvette/96 well flat-bottom plate, transferpettor, desk centrifuge, ice and distilled water.

Procedure:

I. Enzyme preparation:

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

II. **Determination procedure**

1. Preheat Spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 510 nm, set the counter to zero with distilled water.
2. Preheat Reagent 2 in 37℃ water bath for 30 minutes at least.
3. Add reagents as the following:

Reagent name (μL)	Test tube A3	Contrast tube A4	Blank tube A2	Standard tube A1
Distilled water			4	
Standard solution				4
Supernatant	4			
Reagent 1	40	40	40	40
Reagent 2	40	40	40	40
Mix thoroughly, stay in 37℃ for 15 minutes.				
Reagent 3	120	120	120	120
Supernatant		4		
Mix thoroughly, detect absorbance at 510 nm, record as A1, A2, A3, A4.				

III.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) Serum:

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, 204 μL=0.204 mL;

Vs: Supernatant volume, 0.004 mL;

Ve: Extraction volume, 1 mL;

W: Sample weight, g;

T: Reaction time, 15 minutes;

Cpr: Sample protein concentrate, mg/mL.

Note:

1. Reagent 1, Reagent 2 and Reagent 3 should be protected from light.
2. Reagent 3 can not 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. 169, A_C = 0.047, A_B = 0.049,

A_S = 0.449. 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. 169-0.047) ÷ (0.449-0.049)÷0. 1 = 0.509 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. 147, A_C = 0.047, A_B = 0.049, A_S = 0.449. 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. 147-0.047) ÷(0.449-0.049) = 0.0418 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