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: BC2145

Size: 100T/48S

Components:

Extraction reagent: Liquid 60 mL×1 bottle, storage at 2-8℃.

Reagent I: Liquid 5 mL×1 bottle, storage at 2-8℃.

Reagent II: Liquid 5 mL×1 bottle, storage at 2-8℃.

Reagent III: Liquid 15 mL×1 bottle, storage at 2-8℃.

Standard: Liquid 1 mL×1 bottle, 10 μmol/mL phenol standard solution, storage at 2-8℃. Dilute with distilled water to 2.5 μmol/mL before use. The unused reagent shall be sub packed and stored at 2-8 ℃ for 1 week.

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:

1. 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.
2. 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, the spectrophotometer needs to be zeroed with distilled water.
2. Add reagents as the following:

Reagent Name	Test Tube (A3)	Contrast Tube	Blank Tube (A2)	Standard Tube (A1)

Distilled water	-	-	4	-
Standard Solution	-	-	-	4
Supernatant	4	-	-	-
Reagent I	40	40	40	40
Reagent II	40	40	40	40
Mix thoroughly, stay in 37℃ for 15 minutes.				
Reagent III	120	120	120	120
Supernatant	-	4	-	-
Mix thoroughly, detect absorbance at 510 nm, record as A1, A2, A3, A4. The standard tube and blank tube only need to be measured 1-2 times.				

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 milligram 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 gram 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 milliliter 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;
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 I, Reagent II and Reagent III should be protected from light.
2. Reagent III can not be used if it has changed to blue-green.
3. Mix thoroughly quickly after adding Reagent III 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：

BC2020/BC2025	Acetylcholinesterase(AchE) Activity Assay Kit
BC2130/BC2135	Acid Phosphatase(ACP) Activity Assay Kit
BC0840/BC0845	Carboxylesterase(CarE) Activity Assay Kit