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: BC2140

Size: 50T/24S

Components:

Reagent Name	Size	Storage
Extraction reagent	Liquid 30 mL×1	2-8℃
Reagent I	Liquid 10 mL×1	2-8℃
Reagent II	Liquid 10 mL×1	2-8℃
Reagent III	Liquid 30 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Preparation of solution:

1. Standard: 10 μmol/mL phenol standard solution, storage at 2-8℃. Dilute with distilled water to 2.5 μmol/mL before use. (Can absorb 25μL10 μmol/mL phenol standard liquid and 75μL distilled water mixed for use) The unused reagent can be 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, Desk Centrifuge, Transferpettor, 1 mL Glass Cuvette, Ice and Distilled Water.

Procedure:

I. Enzyme preparation:

1. 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.
2. 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	Test Tube	Control Tube	Blank Tube (A_B)	Standard Tube (A_S)
Distilled water	-	-	20	--
Standard solution	-	-	-	20
Supernatant	20	-	-	-
Reagent I	200	200	200	200
Reagent II	200	200	200	200
Mix thoroughly, stay in 37℃ for 15 minutes.				
Reagent III	600	600	600	600
Supernatant	-	20	-	-
Mix thoroughly, detect absorbance at 510 nm, record as A_T, A_C, A_B, A_S. The standard curve and blank tube only need to be measured 1-2 times.				

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) $=[C\times(A_T-A_C) \div(A_S-A_B) \times Vs]\div(Cpr\times Vs)\div T=0.167\times(A3-A4)\div(A1-A2)\div Cpr$

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) $=[C\times(A_T-A_C) \div(A_S-A_B) \times Vs] \div(W\div Ve\times Vs)\div T=0.167\times(A3-A4)\div(A1-A2)\div W$

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) $=[C\times(A_T-A_C) \div(A_S-A_B) \times Vs]\div Vs\div T=0.167\times(A3-A4)\div(A1-A2)$

C: Standard concentration, 2.5 μmol/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 I, Reagent II and Reagent III should be protected from light.
2. Reagent III cannot 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 = 0.324, 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:

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

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