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Tissue and Blood acid phosphatase (ACP)Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0404

Size: 50T/24S

Components:

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

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

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

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

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

Product Description:

In acid condition, ACP catalyze phosphomonoester to inorganic phosphate, which is found in lysozyme of macrophages. ACP can be used in auxiliary diagnosis of prostate cancer.

In acid condition, ACP 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. ACP 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 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. Preheat Reagent 1 in 37℃ water bath for 30 minutes at least.

3. 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			100	

Standard solution				100
Supernatant	100			
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		100		
Mix thoroughly, detect absorbance at 510 nm, record as A1, A2, A3, A4.				

III. ACP activity calculation:

1) Protein concentration:

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

ACP (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 1μmol phenol in the reaction system per minute at 37℃ every g sample.

ACP (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 1μmol phenol in the reaction system per minute at 37℃ every mL serum.

ACP (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, 0.1 mL;

Ve: Extraction reagent 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;
4. ACP is unstable, especially at 37℃ and pH greater than 7, the acid phosphatase is generally required to be prepared on the same day; In the serum sample, add the amount of 10 mg disodium salt sesquihydrate or 5 mg of sodium hydrogen sulfate to 1 mL serum sample. Reduce pH below 6.5. It also can add 2~3 drops of a 30% acetic acid solution to 5 mL serum. and then store at 4℃ for one week.

Experimental example:

1. 0.1 g of liver is added with 1 mL of Extract solution for homogenization. The supernatant is taken and

operated according to the determination steps. The 96 well plate is used to measure and calculate A_T= 0.572, A_C = 0.021, A_B = 0.007, A_S = 0.496.

ACP activity (U/g mass) = 0. 167× (A_T-A_C) ÷ (A_S-A_B) ÷ W = 0. 167× (0.572-0.021) ÷ (0.496-0.007) ÷ 0.1 =1.882 U/g mass.

2. 0.1 g of clover leaves are homogenized and ground by adding 1 mL of Extract solution. The supernatant is taken and operated according to the determination steps. The 96 well plate is used to measure and calculate A_T = 0.074, A_C = 0.023, A_B = 0.007, A_S = 0.496

ACP activity (U/g mass) = 0.167 × (A_T-A_C) ÷ (A_S-A_B) ÷ W =0. 167× (0.074-0.023) ÷ (0.496-0.007) ÷0. 1 =0. 174 U/g mass.

Related Products：

AK0410/AK0409	Acetylcholinesterase(AchE) Activity Assay Kit
AK0402/AK0401	Alkaline Phosphatase(AKP/ALP) Activity Assay Kit
AK0514/AK0513	Carboxylesterase(CarE) Activity Assay Kit