Tissue and Blood Acid Phosphatase (ACP) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC2135

Size: 100T/48S

Components:

Extraction Reagent: Liquid 60 mL×1 bottle, store at 2-8℃.

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

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

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

Standard: Liquid 1 mL×1 bottle, store at 2-8℃, 10 μmol/mL phenol standard solution, dilute with distilled water to 0.625 μmol/mL before use. The unused reagent can be stored at 2-8℃ for 1 week.

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/Microplate Reader, Micro Glass Cuvette/96 Well Flat-Bottom Plate, Transferpettor, Desk Centrifuge, Mortar/Homogenizer, Ice and Distilled Water.

Procedure:

I. Enzyme preparation:

Tissue: Add 1 mL of Extraction reagent to 0.1 g of tissue, grind thoroughly. Centrifuge at 4℃ and 10000 rpm for 10 minutes. Take the supernatant on ice for test.

Serum/Plasma: 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. Preheat Reagent I in 37℃ water bath for 30 minutes at least.
3. Add reagents 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 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	-	20	-	-
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. 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= 0.0417×(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=0.0417×(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=0.0417×(A3-A4)÷(A1-A2)

C: Standard concentration, 0.625 μmol/mL;

Vs: Supernatant volume, 0.02 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 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;
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 1 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= 0.375, A_C = 0.054, A_B = 0.047, A_S = 0.340.

ACP activity (U/g mass) = 0.167× (A_T-A_C) ÷ (A_S-A_B) ÷ W = 0.167 × (0.375-0.054) ÷ (0.340-0.047) ÷ (0.1 = 1.830 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 = 0.079, A_C = 0.051, A_B = 0.047, A_S = 0.340

ACP activity (U/g mass) = 0.167 × (A_T-A_C) ÷ (A_S-A_B) ÷ W = 0.167 × (0.079-0.051) ÷ (0.340-0.047) ÷ (0.1 = 0.160 U/g mass.

Related Products：

BC2020/BC2025	Acetylcholinesterase(AchE) Activity Assay Kit
BC2140/BC2145	Alkaline Phosphatase(AKP/ALP) Activity Assay Kit

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