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: BC2130

Size: 50T/24S

Components:

Reagent Name	Size	Storage
Extraction Reagent	Liquid 30 mL×1 瓶	2-8℃
Reagent I	Liquid 9 mL×1 瓶	2-8℃
Reagent II	Liquid 9 mL×1 瓶	2-8℃
Reagent III	Liquid 18 mL×1 瓶	2-8℃
Reagent IV	Liquid 18 mL×1 瓶	2-8℃
Standard:	Liquid 1 mL×1 支	2-8℃

Preparation of solution：

(1) Standard: 10 μmol/mL phenol standard solution, dilute with distilled water to 0.2 μmol/mL before use. Take 20 μL 10μmol/ml phenol standard solution and 980 μL distilled water mixed for standby. The unused reagent could 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, Desk Centrifuge, Transferpettor, 1 mL Glass Cuvette, Mortar/Homogenizer, Ice and Distilled Water.

Procedure:

I. Enzyme preparation:

Tissue: 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.

Serum/Plasma: 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, the spectrophotometer needs to be zeroed with distilled water.
2. Reagent I is balanced to room temperature before use, and reagent II is preheated at 37 ℃ for 10

min.

3. Add reagents in cuvette as the following:

Reagent Name (μL)	Test Tube (A_T)	Control Tube (A_C)	Blank Tube (A_B)	Standard Tube (A_S)
Supernatant	300	-	-	-
Distilled water	-	-	300	-
Standard solution	-	-	-	300
Reagent I	150	150	150	150
Reagent II	150	150	150	150
Mix thoroughly, stay in 37℃ for 15 minutes.				
Reagent III	300	300	300	300
Reagent IV	300	300	300	300
Supernatant	-	300	-	-
Mix thoroughly, detect absorbance at 510 nm, record as A_T, A_C, A_B, A_S. 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) $=[C\times(A_T - A_C)\div(A_S - A_B)\times Vs]\div(Cpr\times Vs)\div T=0.0133\times(A_T - A_C)\div(A_S - A_B)\div Cpr$

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) $=[C\times(A_T - A_C)\div(A_S - A_B)\times Vs]\div(W\div Ve\times Vs)\div T=0.0133\times(A_T - A_C)\div(A_S - A_B)\div W$

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) $=[C\times(A_T - A_C)\div(A_S - A_B)\times Vs]\div Vs\div T=0.0133\times(A_T - A_C)\div(A_S - A_B)$

C: Standard concentration, 0.2 μmol/mL;

Vs: Supernatant volume, 0.3 mL;

Ve: Extraction reagent volume, 1 mL;

W: Sample weight, g;

T: Reaction time, 15 minutes;

Cpr: Sample protein concentration, mg/mL.

Note:

1. The acid phosphatase samples generally need to be prepared on the same day, and can not be placed at room temperature or pH greater than 7 for too long, easy to inactivate; in the serum sample, 10 mg of disodium hydrogen citrate or 5 mg of sodium hydrogen sulfate can be added to each milliliter of serum to reduce the pH to below 6.5, or 5 mL of serum can be added to 2-3 drops of 30 % acetic acid solution, and stored at 4 °C for 1 week, -20 °C (1 month) or-80 °C (3 months) was the best.
2. If the (A_T-A_C) is greater than 1 or A_T is greater than 1.5, it is recommended to dilute the sample with distilled water for determination; if the (A_T-A_C) is less than 0.01, it is suggested to increase the sample volume or increase the sample volume of the operation table (while reducing the volume of reagent II).

Experimental example:

1. 0.1 g of Rabbit kidney is added with 1 mL of Extract solution for homogenization. The supernatant is taken and operated according to the determination steps. Measure and calculate A_B =0.02, A_S=0.485, A_C=0.051, A4=0.465.

ACP activity (U/g mass) = 0.0133× (A_T -A_C) ÷ (A_S-A_B) ÷ W =0.497 U/g mass.

2. Fetal bovine serum was taken according to the determination steps. Measure and calculate A_B =0.02, A_S=0.485, A_C=0.149, A4=0.525.

ACP activity (U/g mass) = 0.0133× (A_T -A_C) ÷ (A_S-A_B) ÷ W =0.011 U/mL÷0.1 =0.174 U/g mass.

Related Products:

BC2020/BC2025	Acetylcholinesterase(AchE) Activity Assay Kit
BC2140/BC2145	Alkaline Phosphatase(AKP/ALP) Activity Assay Kit
BC0840/BC0845	Carboxylesterase(CarE) Activity Assay Kit