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Acid Phosphatase (ACP) Activity Assay Kit

Catalog No.: BC00045

Size: 120T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email (Sale) order@enkilife.com

✉Email (Techsupport) techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Acid Phosphatase (ACP) Activity Assay Kit
Detection Methods	Colorimetric
Sample type	Serum, plasma, urine, cells, tissues
Detection Type	Enzyme activity
Detection instrument and wavelength	Microplate reader (400-415 nm, optimal detection wavelength 405 nm)
Range	0.2-50 U/L
Sensitivity	0.2U/L

Product Introduction

Acid phosphatase, also known as acid phosphatase, is an acid hydrolase with a high content in lysosomes and is considered to be a marker for identifying lysosomal subcellular components. Acid phosphatase is a family of proteins, and its molecular weight in mammals ranges from 18kD to 100kD. Acid phosphatases are divided into two categories, one is tartrate-sensitive and the other is fluoride-sensitive. Acid phosphatases in lysosomes are tartrate-sensitive, while acid phosphatases in red blood cells and macrophages are fluoride-sensitive. The activity of acid phosphatase in plasma ranges from 2-7.9U/L, and the activity of acid phosphatase in serum ranges from 2.5-11.7U/L. Semen contains high concentrations of acid phosphatase, and the activity can reach 87-436KU/L.

Detection principle

Para-nitrophenyl phosphate (pNPP) is a commonly used chromogenic substrate for phosphatase. Under acidic conditions, para-nitrophenol (*p* -nitrophenol) can be generated by acid phosphatase. Under alkaline conditions, *p* -nitrophenol is a yellow product, and the absorbance can be detected at 400-415nm. The darker the yellow color of the product, the higher the acid phosphatase activity, and vice versa. Based on this, the acid phosphatase activity level can be calculated through colorimetric analysis.

Product composition

serial number	Product Name	Packing Specifications (120T)	How to save

Reagent 1	Assay Buffer	15ml	-20℃
Reagent 2	Chromogenic substrate	2 tubes	Store at -20℃, away from
Reagent 3	*p* -nitrophenol solution	0.1ml	Store at -20℃, away from
Reagent 4	Reaction stop solution	20ml	-20℃
Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage conditions

The unopened kit can be stored at -20℃ for 12 months.

Preparation before the experiment

Sample processing

1. Preparation of cell or tissue lysis buffer: Lyse cells or tissues with appropriate cell or tissue lysis buffer, homogenize appropriately if necessary, and then centrifuge to obtain the supernatant for acid phosphatase detection. Note: The lysis buffer should not contain phosphatase inhibitors. Samples can be frozen at -80°C, but repeated freezing and thawing should be avoided.
2. Preparation of plasma, serum and urine: Plasma and serum can be directly used for the determination of this kit after being prepared according to conventional methods. However, in order to eliminate the interference of the color of the sample itself, a control with plasma or serum but no substrate should be set up. Anticoagulant tubes containing EDTA and citrate cannot be used when preparing plasma. Urine can also be used directly for determination. The above samples can be frozen at -80℃, but repeated freezing and thawing should be avoided.
3. Dilution of samples: If the sample contains highly active acid phosphatase, it can be diluted with the original lysis buffer or PBS, or with the detection buffer in the kit. If the detection buffer provided in the kit is used for dilution, it is necessary to keep enough detection buffer for the detection process of the kit.

· Preparation of the kit

Take out all reagents and return to room temperature before use.

1. Colorimetric substrate solution: Take a tube of colorimetric substrate and dissolve it in 2.5 ml of detection buffer (you can dissolve it with 1 ml of detection buffer first, and after fully dissolving and mixing, transfer it to a 15 ml centrifuge tube and add 1.5 ml of detection buffer), fully dissolve and mix, and place it on ice. Freshly prepared colorimetric substrate solution must be used within 6 hours.

2. Standard working solution: Take 10μl *of p* -nitrophenol solution (10mM) and dilute to 0.2ml with assay buffer, with a final concentration of 0.5mM.

Operation Process

1. Refer to the table below to set up blank control wells, standard wells, and sample wells using a 96-well plate. The dosage of the standard is 4, 8, 16, 24, 32, and 40 μl, respectively. The sample can usually be added directly to 40 μl. If the acid phosphatase activity in the sample is too high, the sample dosage can be reduced or diluted appropriately before measurement. It is best to set up parallel wells or triplicate wells for sample testing.

	Blank	Standard	Sample
Assay Buffer	40μl	(80-x) μl	(40-y) μl
Chromogenic substrate	40μl	--	40μl
Sample	--	--	yμl
Standard Working Solution	--	xμl	--

2. Mix by gently blowing up and down with a pipette tip, or by using a shaker.
3. Incubate at 37°C for 5-10 minutes. (Note: When the acid phosphatase activity in the sample to be tested is low, the incubation time can be appropriately extended to 30 minutes)
4. Add 100μl of stop solution to each well to terminate the reaction. At this point, the standard or wells with acid phosphatase activity will show different shades of yellow.
5. Measure the absorbance at 405nm. If you cannot measure at 405nm, you can also measure the absorbance in the range of 400-415nm. If you cannot measure immediately, you can complete the measurement within a few hours, and the yellow color that appears is stable within a few hours.

Result Calculation

1. the amount of acid phosphatase required to hydrolyze para-nitrophenyl phosphate chromogenic substrate to produce 1 micromole of *p -nitrophenol per minute is defined as one enzyme activity unit.*
2. According to the definition of enzyme activity, the acid phosphatase activity in the sample was calculated.

Notes

1. If you want to perform absolute quantification of enzyme activity, you must pay attention to accurate timing when performing the enzyme reaction. At this time, it is recommended to use a

longer incubation time such as 30 minutes to reduce the time error during the operation. At the same time, if the enzyme activity in the sample is high, the sample can be appropriately diluted in advance.

2. The presence of various acid phosphatase inhibitors must be avoided in the sample solution.
3. A tube of colorimetric substrate must be used up on the same day after preparation, so please prepare more samples for testing together to avoid wasting the kit.
4. *p* -nitrophenol solution is harmful to the human body. Please be careful when handling it and take effective protection to avoid direct contact with the human body or inhalation. The reaction termination solution is corrosive. Please be careful when handling it and take effective protection to avoid direct contact with the human body or corrosion of other items.
5. This product is limited to scientific research by professionals and must not be used for clinical diagnosis or treatment, used as food or medicine, or stored in ordinary residences.