(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Alkaline Phosphatase (ALP) Activity Assay Kit

Catalog No.: BC00046

Size: 100T

Please read the instructions carefully before use. 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

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

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	Alkaline Phosphatase (ALP) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Serum,Plasma, Urine, Cells, Tissues
Assay Type	Enzyme activity
Detection Instrument	Microplate reader(400-415nm,optimal detection wavelength 405nm)

Product Introduction

Alkaline phosphatase (AP/ALP/AKP/ALKP/ALPase/Alk Phos), also known as alkaline phosphatase (EC 3.1.3.1), can catalyze the hydrolysis of phosphate bonds under alkaline conditions. In mammals, the activity of alkaline phosphatase in the liver, bile duct, kidney, bone and placenta is relatively high. Common alkaline phosphatases include intestinal alkaline phosphatase (ALPI), non-tissue-specific alkaline phosphatase (ALPL) and placental alkaline phosphatase (PLAP). Except for placental alkaline phosphatase (placental isoform), other endogenous alkaline phosphatases are easily inactivated after heating. The common Calf Intestinal Alkaline Phosphatase (CIAP/CIP) is widely used for labeling secondary antibodies and ultimately for the detection of proteins and nucleic acids. It is also commonly used for dephosphorylation of the 5′ and 3′ ends of DNA or RNA, especially dephosphorylation of the 5′ end of plasmids to avoid plasmid self-ligation.
In stem cells, such as iPS, alkaline phosphatase activity is very high and is often used as a marker of successful iPS induction. In addition, the activity of alkaline phosphatase in differentiated colon cancer cells will also increase significantly, which is used as a qualitative and quantitative indicator of the degree of differentiation of colon cancer cells. In addition, the increase in serum alkaline phosphatase, called hyperalkaline phosphatasemia, is considered to be related to malignant biliary obstruction, primary biliary cirrhosis, Hepatobiliary diseases such as primary sclerosing cholangitis, hepatic lymphoma and hepatic sarcoidosis are closely related. Elevated serum alkaline phosphatase activity is also closely related to bone formation, because alkaline phosphatase is a by-product of osteoblasts. Low serum alkaline phosphatase activity is also associated with some diseases. The alkaline phosphatase activity in the serum of children and pregnant women is higher than that of ordinary people. The alkaline phosphatase activity in serum ranges from 20-140U/L.

Principle

Para-nitrophenyl phosphate (pNPP) is a commonly used chromogenic substrate for phosphatase. Under alkaline conditions, it can generate para-nitrophenol (pNP) under the action of alkaline phosphatase. *p*- nitrophenol is a yellow product under alkaline conditions, and the absorbance can be detected at 400-415nm. The darker the yellow color of the

product, the higher the activity of alkaline phosphatase, and vice versa. Based on this, the activity level of alkaline phosphatase can be calculated by colorimetric analysis.

Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Assay Buffer	15ml	-20℃
Reagent 2	Chromogenic Substrate	2 tubes	-20℃, keep away from light.
Reagent 3	*p* -nitrophenol solution (10mM)	0.1ml	-20℃, keep away from light.
Reagent 4	Reaction stop solution	12ml	-20℃
Consumable 1	Microplate(96 wells)	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

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

Experimental Preparation

● 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 alkaline 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 usually also be used directly for determination. The above samples can be frozen at -80°C, but repeated freezing and thawing should be avoided.
3. Dilution of samples: If the sample contains highly active alkaline 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 assay 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 *p*- nitrophenol solution (10mM) and dilute to 0.2ml with assay buffer, the final concentration is 0.5mM.

Operation process

1. Refer to the table below to set up blank control wells, standard wells, and sample wells in a 96-well plate. The dosage of the standard is 4, 8, 16, 24, 32, and 40 μl, respectively. Samples can usually be added directly to 50 μl. If the alkaline phosphatase activity in the sample is too high, the sample dosage can be reduced or diluted appropriately before measurement.

	Blank	Standard	Sample
Assay Buffer	50μl	(100-x) μl	(50-y) μl
Chromogenic substrate	50μl	--	50μl
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 alkaline 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 alkaline 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. Definition of alkaline phosphatase activity unit: In diethanolamine (DEA) buffer at pH 9.8, at 37°C, the amount of alkaline phosphatase required to hydrolyze para-nitrophenyl phosphate chromogenic substrate to produce 1 μM of *p*- nitrophenol per minute is defined as one enzyme activity unit, also known as one DEA enzyme activity unit. In glycine buffer at pH 9.6, at 25°C, the amount of alkaline phosphatase required to hydrolyze para-nitrophenyl phosphate chromogenic substrate to produce 1 μM of *p*- nitrophenol per minute is defined as one enzyme activity unit, also known as one Glycine enzyme activity

unit. One Glycine enzyme activity unit is approximately equivalent to 3 DEA enzyme activity units. This kit measures DEA enzyme activity units.
2. According to the definition of enzyme activity, the alkaline 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. Inhibitors of alkaline phosphatase such as EDTA, fluoride ions, and citrate must be avoided in the sample solution.
3. The detection buffer and *p*- nitrophenol solution are harmful to the human body. Please be careful when handling them 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 them and take effective protection to avoid direct contact with the human body or corrosion of other items.
4. This product is for Research Use Only and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.