Alkaline Phosphatase (ALP) Activity Assay Kit

Catalogue No.: K099

Size: 96T(80 samples)

Range: 0.13-50 King unit/100mL

Sensitivity: 0.13 King unit/100mL

Kit component:

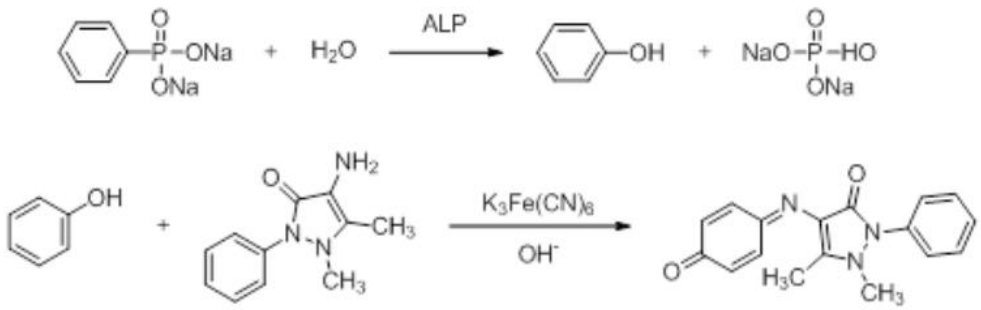
Item	Component	Size（96T）	Storage
Reagent 1	Buffer Solution	3 mL	2-8℃ shading light
Reagent 2	Substrate Solution	3 mL	2-8℃ shading light
Reagent 3	Chromogenic Agent	18 mL	2-8℃ shading light
Reagent 4	0.5 mg/mL Phenol Standard	1.5 mL	2-8℃ shading light
	Product Description	1 copy	2-8℃ shading light

Storage:

The kit should be stored at 2-8℃ shading light for 6 months.

Principle of the Assay:

Alkaline phosphatase decompose benzene disodium phosphate to produce free phenol and phosphoric acid. Phenol react with 4-aminopyrline in alkaline solution and oxidizes with potassium ferricyanide to form red quinone derivative. The enzyme activity can be calculated indirectly by measuring the OD value.



This kit can be used to measure alkaline phosphatase (ALP) activity in serum (plasma), tissue, cells and other samples.

Assay Procedure (For reference):

1. **Reagent preparation**

a. Bring all reagents to room temperature before use.

b. The preparation of working solution:

Mix reagent 1 and reagent 2 thoroughly at the volume ratio of 1:1. The working solution should be prepared on spot, and the unused working solution can be stored at 2-8°C for 1 day away from light.

c. The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 0.5 mg/mL phenol standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/mL)	0	0.025	0.05	0.1	0.2	0.3	0.4	0.5
0.5 mg/mL phenol standard (μL)	0	5	10	20	40	60	80	100
Double distilled water (μL)	100	95	90	80	60	40	20	0

2. Sample preparation

a. **Preparation of sample**

Serum and plasma: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (K001).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300-500 μL normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (K001).

b. **Dilution of sample**

Before the formal test, 2-3 samples with significant differences should be selected and diluted into different concentrations for pre-test. The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human urine	1
Rat serum	1
Cells culture supernatan	1
10% Mouse kidney tissue homogenate	30-50
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
HepG2 cells	1

Note: The diluent is saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

3. Add samples and test

① Standard well: add 5 μL of standards with different concentrations to the corresponding wells.

Sample well: add 5 μL of sample to the corresponding wells.

② Add 50 μL of working solution and mix fully for 30 s with microplate reader.

③ Incubate at 37℃ for 15 min, then add 150 μL of chromogenic agent immediately, mix fully.

④ Measure the OD values of each well at 520 nm with microplate reader.

Calculation:

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of 1 mg phenol produced by 100 mL sample react with the substrate in 15 min is defined as 1 ALP activity unit.

$$\text{ALP activity (King unit/100 mL)} = (\Delta A\text{-}b) \div a \times V_1 \times f$$

2. Tissue and cells sample:

Definition: The amount of 1 mg phenol produced by 1 g tissue protein react with the substrate in 15 min is defined as 1 ALP activity unit.

$$\text{ALP activity (King unit/gprot)} = (\Delta A - b) \div a \div C_{pr} \times f$$

Note:

ΔA: Absolute OD (ODSample – ODBlank).

V1: The volume of sample in definition, 100 mL.

f: Dilution factor of sample before test.

Cpr: Concentration of protein in sample, gprot/mL.

Notes:

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.

2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.

3. Protection methods must be taken by wearing a lab coat and latex gloves during the experiment.

4. The detection range of the kit is not equivalent to the concentration range of the substance to be measured in the sample. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.

5. If the samples to be tested are not among the types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.

6. The experimental results are closely related to the situation of reagents, operations, environment and so on. FineTest will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.