

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

**Catalog No: E-BC-K094-S**

**Specification: 50 assays(48 samples)/100 assays(96 samples)**

**Measuring instrument: Spectrophotometer (520 nm)**

**Detection range: 0.27-40 U/100 mL**

## **Elabscience® Acid Phosphatase (ACP) Activity Assay Kit**

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help:

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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**Assay summary**

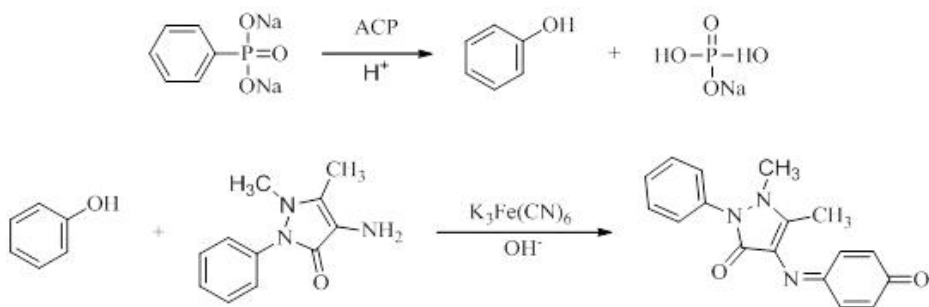


## Intended use

This kit can be used for detection of ACP activity in serum, plasma, urine, tissue and cells sample.

## Detection principle

Acid phosphatase decomposes disodium phenyl phosphate under acidic conditions to produce free phenol and phosphoric acid. Phenol acts with 4-aminoantipyrine in alkaline solution, and oxidizes to a derivative of red quinone by potassium ferricyanide. The activity of the ACP can be calculated by measuring the OD value at 520 nm.



## Kit components & storage

| Item      | Component               | Size 1<br>(50 assays) | Size 2<br>(100 assays) | Storage                       |
|-----------|-------------------------|-----------------------|------------------------|-------------------------------|
| Reagent 1 | Buffer Solution         | 30 mL ×1 vial         | 60 mL × 1 vial         | 2-8°C, 12 months              |
| Reagent 2 | Substrate Solution      | 30 mL ×1 vial         | 60 mL × 1 vial         | 2-8°C, 12 month shading light |
| Reagent 3 | Alkali Reagent          | 60 mL ×1 vial         | 60 mL × 2 vials        | 2-8°C, 12 month shading light |
| Reagent 4 | Chromogenic Agent       | 60 mL ×2 vials        | 60 mL × 3 vials        | 2-8°C, 12 month shading light |
| Reagent 5 | 1 mg/mL Phenol Standard | 1 mL ×1 vial          | 1 mL × 1 vial          | 2-8°C, 12 month shading light |

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

## Materials prepared by users

### Instruments:

Spectrophotometer (520 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

### Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

## Reagent preparation

- ① Equilibrate all reagents to room temperature before use.
- ② The preparation of 0.1 mg/mL phenol standard application solution:  
For each well, prepare 50  $\mu\text{L}$  of 0.1 mg/mL phenol standard application solution (mix well 5  $\mu\text{L}$  of 1 mg/mL phenol standard and 45  $\mu\text{L}$  of double distilled water). The 0.1 mg/mL phenol standard application solution should be prepared on spot.

## Sample preparation

### ① Sample preparation

**Serum and plasma:** detect directly. If not detected on the same day, the serum or plasma can be stored at  $-80^{\circ}\text{C}$  for a month.

### **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  $\mu\text{L}$  normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at  $4^{\circ}\text{C}$ .
- ④ Centrifuge at  $10000\times g$  for 10 min at  $4^{\circ}\text{C}$  to remove insoluble material. Collect supernatant and keep it on ice for detection.
- ⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

### **Cell (adherent or suspension) samples:**

- ① Harvest the number of cells needed for each assay (initial recommendation  $1\times 10^6$  cells).
- ② Wash cells with PBS (0.01 M, pH 7.4).
- ③ Homogenize  $1\times 10^6$  cells in 300-500  $\mu\text{L}$  normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at  $4^{\circ}\text{C}$ .
- ④ Centrifuge at  $10000\times g$  for 10 min at  $4^{\circ}\text{C}$  to remove insoluble material. Collect supernatant and keep it on ice for detection.
- ⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M)

## ② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

| Sample type                            | Dilution factor |
|--|-----------------|
| Human milk                             | 1               |
| Human saliva                           | 1               |
| Human urine                            | 1               |
| Human serum                            | 1               |
| HepG2 cells homogenization             | 2-8             |
| 10% Mouse kidney tissue homogenization | 8-12            |
| 10% Mouse liver tissue homogenization  | 8-12            |
| 10% Mouse spleen tissue homogenization | 8-12            |

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

## Operating steps

- ① Blank well: add 50  $\mu\text{L}$  of double distilled water into a 5 mL EP tube.  
Standard well: add 50  $\mu\text{L}$  of 0.1 mg/mL phenol standard application solution into a 5 mL EP tube.  
Sample well: add 50  $\mu\text{L}$  of sample into a 5 mL EP tube.
- ② Successively add 500  $\mu\text{L}$  of buffer solution and 500  $\mu\text{L}$  of substrate solution respectively and oscillate fully with the vortex mixer.
- ③ Incubate at 37  $^{\circ}\text{C}$  for 30 min, then add 1000  $\mu\text{L}$  of alkali reagent and 1500  $\mu\text{L}$  of chromogenic agent immediately, oscillate fully with the vortex mixer and stand at room temperature for 10 min.
- ④ Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 520 nm with 1 cm optical path quartz cuvette.



## Calculation

**The sample:**

### 1. Serum (plasma) sample:

**Definition:** The amount of 1 mg phenol produced by 100 mL sample react with the substrate in 30 min is defined as 1 unit.

$$\text{ACP activity} \frac{\Delta A_1}{(\text{U}/100 \text{ mL})} = \frac{\Delta A_1}{\Delta A_2} \times m \times \frac{V_1}{V} \times f$$

### 2. Tissue and cells sample:

**Definition:** The amount of 1 mg phenol produced by 1 g tissue protein react with the substrate in 30 min is defined as 1 unit.

$$\text{ACP activity} \frac{\Delta A_1}{(\text{U}/\text{gprot})} = \frac{\Delta A_1}{\Delta A_2} \times m \div (C_{\text{pr}} \times V) \times f$$

### [Note]

$\Delta A_1$ :  $\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}$ .

$\Delta A_2$ :  $\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}$ .

m: Phenol content of standard tube, 0.005 mg.

$C_{\text{pr}}$ : Protein concentration of tested sample, gprot/mL.

V: The volume of sample, 0.05 mL.

$V_1$ : The volume of sample in definition, 100 mL.

f: Dilution factor of sample before test.

## Appendix I Performance Characteristics

### 1. Parameter:

#### Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

| Parameters      | Sample 1 | Sample 2 | Sample 3 |
|-----------------|----------|----------|----------|
| Mean (U/100 mL) | 2.80     | 16.50    | 32.40    |
| %CV             | 2.9      | 2.6      | 2.9      |

#### Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters      | Sample 1 | Sample 2 | Sample 3 |
|-----------------|----------|----------|----------|
| Mean (U/100 mL) | 2.80     | 16.50    | 32.40    |
| %CV             | 7.0      | 6.9      | 7.4      |

#### Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

|                           | Sample 1 | Sample 2 | Sample 3 |
|---------------------------|----------|----------|----------|
| Expected Conc. (U/100 mL) | 10.8     | 22.5     | 35.5     |
| Observed Conc. (U/100 mL) | 10.7     | 22.1     | 36.6     |
| recovery rate(%)          | 99       | 98       | 103      |

#### Sensitivity

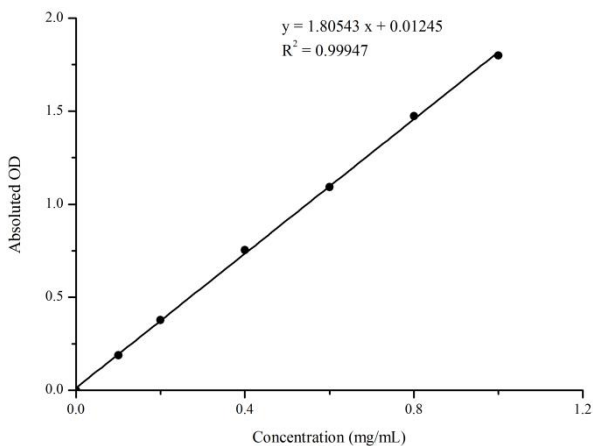
The analytical sensitivity of the assay is 0.27 U/100 mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

## 2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

| Concentration (mg/mL) | 0     | 0.1   | 0.2   | 0.4   | 0.6   | 0.8   | 1     |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|
| Average OD            | 0.018 | 0.206 | 0.396 | 0.772 | 1.110 | 1.491 | 1.817 |
| Absoluted OD          | 0     | 0.188 | 0.378 | 0.754 | 1.092 | 1.473 | 1.799 |



## Appendix Π Example Analysis

### Example analysis :

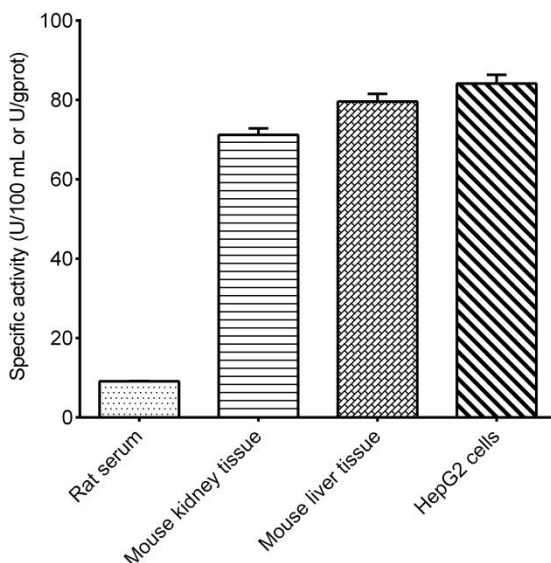
Take 50  $\mu$ L of rat serum, carry the assay according to the operation steps.

The results are as follows:

The average OD value of the sample is 0.200, the average OD value of the blank is 0.037, the average OD value of the standard is 0.215, and the calculation result is:

$$\text{ACP activity (U/100 mL)} = \frac{0.200-0.037}{0.215-0.037} \times 0.005 \times \frac{100}{0.05} = 9.16 \text{ U/100 mL}$$

Detect rat serum, 10% mouse kidney tissue homogenate (the concentration of protein in sample is 0.007 gprot/mL, dilute for 10 times), 10% mouse liver tissue homogenate (the concentration of protein in sample is 0.013 gprot/mL, dilute for 10 times), HepG2 cells (the concentration of protein in sample is 0.005 gprot/mL, dilute for 5 times) according to the protocol, the result is as follows:



## Statement

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 lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience 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.





