# CheKine™ Tissue and Blood Alkaline Phosphatase（AKP/ALP）Colorimetric Assay Kit (Version 2020)

（Cat# KTB1700，48/96 T，Colorimetric, Quantitative, Store at -20°C, protect from light, valid for 1 year）

**Introduction**：AKP / ALP is a zinc-containing glycoprotease that can hydrolyze various natural and synthetic phospholipid monoester compounds in an alkaline environment. AKP / ALP is widely distributed in various organs of the human body, mainly the liver.

In an alkaline environment, AKP / ALP catalyzes phthalate disodium to generate free phenol; phenol reacts with 4-aminoantipyrine and potassium ferricyanide to produce a red quinone derivative, which has characteristic light absorption at 510nm; the absorbance increase rate reflects AKP activity.

**Application:** Tissue sample lysate or the supernatant of homogenized product, plasma, serum, urine or purified enzyme sample, etc.

**Kit Components and Storage Conditions:**

| Kit Components | Quantity | | Storage Conditions |
|---|---|---|---|
| | 48 T | 96 T | |
| Extraction Buffer | 50 mL | 100 mL | 4°C |
| Chromogen A | 2.5 mL | 5 mL | 4°C, protect from light |
| Chromogen B | 2.5 mL | 5 mL | 4°C, protect from light |
| Chromogen C | 7.5 mL | 15 mL | 4°C, protect from light. Do not use if its color change to blue-green |
| Standard | 0.5 mL | 0.5 mL | （2 μmol/mL Phenol Standard Solution），4°C |

**Other Materials Required, Not Supplied**：Standard microplate reader (capable of measuring absorbance at 510 nm), Centrifuge, Incubator, Ice Maker, Precision pipettes and disposable pipette tips, Distilled or deionized water, Assorted glassware for the preparation of reagents and buffer solutions, 96 microwell plate, Dounce homogenizer (for tissue samples).

## Assay Protocol

### Sample Preparation:

Note: All samples can be stored at -80°C for 1 month, but avoid repeated freezing and thawing. If the sample contains higher active alkaline phosphatase, it can be diluted with Extraction Buffer or PBS.

1. Tissue: Weigh about 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice; centrifuge at 10000 rpm 4°C for 10 min, take the supernatant and place on ice to be tested.

2. Serum (plasma) and urine samples: Plasma and serum can be directly measured after being prepared according to conventional methods, but in order to eliminate the sample color interference, a control group needs to be set to add plasma or serum without adding substrate. EDTA and citrate cannot be used in plasma preparation, and other anticoagulants can be used. Urine can be used directly for measurement.

### Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 510 nm.

2. Preheat Chromogen B in a 37°C water bath for more than 30 min.

3. Blank tube: Take EP tube, add 4 μL distilled water, 40 μL Chromogen A, 40 μL Chromogen B, mix and place in 37°C water bath for 15 min; add Chromogen C 120 μL, mix and measure absorbance at 510 nm, record as A blank tube.

4. Standard tube: Take EP tube, add 4 μL standard, 40 μL Chromogen A, 40 μL Chromogen B, mix and place in 37°C water bath for 15 min; add Chromogen C 120 μL, mix and measure absorbance at 510 nm, record as A standard tube.

5. Control tube: Take EP tube, add 40 μL Chromogen A, 40 μL Chromogen B, mix and place in 37°C water bath for 15 min; add Chromogen C 120 μL, mix; finally add 4 μL supernatant, mix at 510 nm, record as A control tube.

6. Test tube: Take EP tube, add 4 μL supernatant, 40 μL Chromogen A, 40 μL Chromogen B, mix and place in 37°C water bath for 15 min; add Chromogen C 120 μL, mix and measure absorbance at 510 nm, record as A test tube.

In a measurement, it only needs one standard tube and blank tube, each sample needs test tube and control tube.

7. According to the enzyme activity definition, calculate the alkaline phosphatase activity in the sample.

### Calculation of Results:

Calculation of AKP/ALP Active Unit

96-well plates calculation formula as below

1. Calculated by protein concentration

Active unit definition: At 37°C,1 μmol phenol produced per min in 1 mg protein reaction system is defined as a unit of enzyme activity.

AKP/ALP(U/mgprot)=[C standard×(A test tube-A control tube)÷(A standard tube-A blank tube)×V sample]÷(Cpr×V sample)÷T =0.133×(A test tube -A control tube)÷(A standard tube -A blank tube)÷Cpr

2. Calculated by sample fresh weight

Active unit definition: At 37°C,1 μmol phenol produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

AKP/ALP(U/g fresh weight)=[C standard×(A test tube -A control tube)÷(A standard tube -A blank tube)×V sample] ÷(W÷V extract ×V sample)÷T=0.133×(A test tube -A control tube)÷(A standard tube -A blank tube)÷W

3. Calculated by solution volume

Active unit definition: At 37°C,1 μmol phenol produced per min in 1 mL blood reaction system is defined as a unit of enzyme activity.

AKP/ALP(U/mL)=[C standard×(A test tube -A control tube)÷(A standard tube -A blank tube)×V sample] ÷V sample ÷T =0.133×(A test tube -A control tube)÷(A standard tube -A blank tube)

C standard: 2μmol/mL; V total: total reaction volume（mL）, 204 μL=0.204 mL; V sample: supernatant volume added to the reaction system（mL）, 0.004 mL; T: reaction time（min）, 15 min; V extract: extract solution added, 1 mL; W: sample fresh weight, g; Cpr: supernatant protein concentration（mg/mL）, measurement is needed, it is recommended to use Abbkine *Protein Quantification Kit (BCA Assay)*（#KTD3001.

**Related Products Recommends:**

CheKine™ Xanthine Oxidase Assay Kit#KTB1070

CheKine™ Catalase (CAT) Activity Assay Kit#KTB1040

CheKine™ Lactate Dehydrogenase (LDH) Assay Kit#KTB1110

**Precautions**

1. Assay kit is intended for research use only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

2. Do not mix or substitute kit reagents or materials from other lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

3. Avoid foaming or bubbles when mixing or reconstituting components.

4. Avoid cross-contamination, change pipette tips between additions of standards, samples and reagents. Also, use separate reservoirs for each reagent.

5. Ensure all reagents and equipment are at the appropriate temperature before assay.

6. Chromogen A, Chromogen B and Chromogen C should be kept away from light. Chromogen C cannot be used after it turns blue-green.

7. After adding Chromogen C, it must be mixed immediately, otherwise the color development will not be complete.