CheKine™ Micro Tissue and Blood Alkaline Phosphatase (AKP/ALP) Assay Kit

Cat #: KTB1700 Size: 48 T/96 T

	Micro Tissue and Blood Alkaline Phosphatase (AKP/ALP) Assay Kit		
REF	**Cat #:** KTB1700	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal Tissues, Urines		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Alkaline Phosphatase (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 510 nm; the absorbance increase rate can reflect AKP/ALP activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Chromogen A	2.5 mL	5 mL	4℃, protected from light
Chromogen B	2.5 mL	5 mL	4℃, protected from light
Chromogen C	7.5 mL	15 mL	4℃, protected from light
Standard	0.5 mL	0.5 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 510 nm
- Incubator, ice maker, freezing centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Dounce homogenizer(for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Chromogen A: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Chromogen B: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Chromogen C: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 rpm for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Serum or Plasma: Plasma and serum can be directly measured. EDTA and citrate cannot be used in plasma preparation, and other anticoagulants can be used.

3. Urines: Tested directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 510 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Operate according to the sample addition and reaction process in the following table

Reagent	Blank Well(μL)	Standard Well(μL)	Control Well(μL)	Test Well (μL)
Deionized Water	4	0	0	0
Standard	0	4	0	0
Sample	0	0	0	4
Chromogen A	40	40	40	40
Chromogen B	40	40	40	40
Mix and place in 37℃ incubation for 15 min				
Chromogen C	120	120	120	120
Sample	0	0	4	0
Mix and measure absorbance at 510 nm, it only needs one standard well and blank well. Every sample needs to set a control well. The absorbance of each tube recorded as A_{Blank}, $A_{Standard}$, $A_{Control}$, A_{Test}, respectively.				

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculated by protein concentration

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

AKP/ALP (U/mg prot)=$[C_{Standard}\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})\times V_{Sample}]\div(Cpr\times V_{Sample})\div T$

$=0.133\times(A_{Test}-A_{Control})\div(A_{Standard}-A_{Blank})\div Cpr$

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}-$A_{Control}$)÷($A_{Standard}$-A_{Blank})×V_{Sample}]÷(W÷$V_{Extract}$×V_{Sample})÷T

=0.133×(A_{Test}-$A_{Control}$)÷($A_{Standard}$-A_{Blank})÷W

3. Calculated by solution volume

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

AKP/ALP (U/mL)= [$C_{Standard}$×(A_{Test}-$A_{Control}$)÷($A_{Standard}$-A_{Blank})×V_{Sample}]÷V_{Sample}÷T

=0.133×(A_{Test}-$A_{Control}$)÷($A_{Standard}$-A_{Blank})

Where: $C_{Standard}$: Concentration of Standard; 2 μmol/mL; V_{Sample}: Supernatant volume added to the reaction system, 0.004 mL; T: Reaction time, 15 min; $V_{Extract}$: Extract solution added, 1 mL; W: Sample fresh weight, g; Cpr: Supernatant protein concentration, mg/mL.

Recommended Products

Catalog No.	Product Name
KTB1070	CheKine™ Micro Xanthine Oxidase Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.