Ver.240703

Tissue and Blood Alkaline Phosphatase (AKP/ALP) Activity Assay Kit

BC8803-02 (100 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Alkaline Phosphatase (AKP/ALP) is a zinc containing glycoprotease, which hydrolyses various natural and synthetic phospholipid monoester compounds in alkaline condition. AKP/ALP is widely distributed in human organs, mainly in liver.

In alkaline condition, AKP/ALP catalyses hydrolysis of disodium phenyl phosphate to phenol and the phenol reacts with 4-aminoantipyrine and potassium ferrycyanide to form red quinone derivative with an absorbance at 510nm. AKP/ALP activity can be calculated from the rate of increase in absorbance at 510nm.

Kit components

Reagent	Volume	Storage
Extraction Reagent	60mL	4°C
Reagent I	5mL	4°C
Reagent II	5mL	4°C
Reagent III	15mL	4°C
Standard	1mL Phenol standard (10μmol/ml)	4°C
Dilute the phenol standard with distilled water to 2.5μmol/ml before use.		

Note: Unused phenol standard can be stored for 1 week at 4°C

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat bottom plate and distilled water.

Enzyme Preparation

Add 1mL Extraction Reagent to 0.1g tissue, grind thoroughly. Centrifuge at 10000rpm, 4°C for 10 minutes. Take the supernatant on ice for the assay. Blood sample can be directly used for detection. Dilute the sample with Extraction Reagent if the reading is high.

Operation Procedures

1. Preheat the spectrophotometer/microplate reader for 30 min, adjust wavelength to 510 nm and set zero with distilled water.
2. Add reagents to 1.5ml tube as follows

Reagent	Standard (A1)	Blank (A2)	Test (A3)	Control (A4)
Distilled Water	-	4µL	-	-
Standard solution	4µL	-	-	-
Sample	-	-	4µL	-
Reagent I	40µL	40µL	40µL	40µL
Reagent II	40µL	40µL	40µL	40µL
Mix thoroughly and incubate at 37°C for 15 minutes				
Reagent III	120µL	120µL	120µL	120µL
Sample	-	-	-	4µL
Mix thoroughly and measure absorbance at 510nm. Record absorbance as A1, A2, A3 and A4.				

Calculations

1. ***Protein concentration:***
   Unit definition: One unit of enzyme activity is defined as the amount of enzyme which generates of 1µmol phenol in the reaction system per minute at 37°C for every milligram of protein.
   AKP/ALP (U/mg Prot) = [C × (A3 – A4) ÷ (A1 – A2) × Vs] ÷ (Cpr × Vs) ÷ T
   = 0.167 × [(A3-A4) ÷ (A1-A2)] ÷ Cpr
2. ***Sample weight:***
   Unit definition: One unit of enzyme activity is defined as the amount of enzyme which generates of 1µmol phenol in the reaction system per minute at 37°C for every gram of tissue.
   AKP/ALP (U/g weight) = [C × (A3 – A4) ÷ (A1 – A2) × Vs] ÷ (W ÷ Ve × Vs) ÷ T
   = 0.167 × [(A3-A4) ÷ (A1-A2)] ÷ W
3. ***Blood:***
   Unit definition: One unit of enzyme activity is defined as the amount of enzyme which generates of 1µmol phenol in the reaction system per minute at 37°C for every millilitre of serum.
   AKP/ALP (U/mL) = [C × (A3 – A4) ÷ (A1 – A2) × Vs] ÷ Vs ÷ T
   = 0.167 × [(A3-A4) ÷ (A1-A2)]

Cpr : Sample protein concentration (mg/ml)
W : Sample weight in grams.
C : Standard concentration, 2.5 µmol/mL
Vs: : Sample volume, 0.004mL
Ve : Extraction volume, 1mL
T : Reaction time, 15 minutes