Alkaline Phosphatase Activity

ColorimetricMicroplate Assay Kit

User Manual

Catalog # CAK1003

(Version 1.4E)

Detection and Quantification of Alkaline Phosphatase (ALP) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in an alkaline environment, resulting in the formation of an organic radical and inorganic phosphate. In mammals, this enzyme is found mainly in the liver and bones. Marked increase in serum ALP levels, a disease known as hyperalkalinephosphatasemia, has been associated with malignant biliary obstruction, primary biliary cirrhosis, primary sclerosing cholangitis, hepatic lymphoma and sarcoidosis.

Alkaline Phosphatase Activity ColorimetricMicroplate Assay Kit provides a simple and direct procedure for measuring alkalinephosphatase activity in a variety of samples.The assay is initiated with the enzymatic hydrolysis of the disodium phenyl phosphate by alkalinephosphatase. The enzyme catalysed reaction products can be measured at a colorimetric readout at 510 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	4 mlx 1	4 °C, keep in dark
Substrate	Powder x 1	4 °C, keep in dark
Dye Reagent I	Powder x 1	4 °C, keep in dark
Dye Reagent II	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Positive Control	1µlx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 510 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml distilled waterto generate 100 mmol/L of standardstock solution,keep in dark andstore at 4 °C for 1-2 weeks or -20°C for 3-4 monthsafter reconstitution.Then dilute to5mmol/Lstandard top solutionby adding50 µlstock solution into 950 µldistilled water**.**Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be5/2.5/1.25/0.625/0.312/0.156/0.078mmol/L.

Substrate: Add 4 ml distilled water to dissolve before use.Keep in dark for immediateuse or store at -20°C for 1 week after reconstitution.

Dye Reagent I: Add 9 ml distilled water to dissolve before use.Keep in dark for immediate use or store at -20°C for 1-2weeks after reconstitution.

Dye Reagent II: Add 2 ml distilled water to dissolve before use.Keep in dark and store at 4 °C for 3-4 weeks after reconstitution.

Positive Control: add 1 ml Assay Buffer to dissolve before use, mix.Store at -80 °C for 2-3 months after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly, or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent*	Sample**	Standard	Blank	Positive Control
Sample	10µl	--	--	--
Standard	--	10µl	--	--
Distilled water	--	--	10µl	--
Positive Control	--	--	--	10µl
Reaction Buffer	40 µl	40 µl	40 µl	40 µl
Substrate	40 µl	40 µl	40 µl	40 µl
Mix, put it in the oven,37 °C for 15 minutes.				
Dye Reagent I	90 µl	90 µl	90 µl	90 µl
Dye Reagent II	20 µl	20 µl	20 µl	20 µl
Mix,wait for 10 minutes, record absorbance measured at 510 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VI. CALCULATION

Unit Definition: One unit of Alkaline Phosphatase activity is defined as the enzyme generates 1 μmolphenol per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Blank}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times C_{Protein}\times V/ (V + V_{Assay})\times T}(U/mg)$$

2.2 According to the weight of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample}/ V_{Assay})\times T}(U/g)$$

2.3 According to the volume of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times V / (V + V_{Assay})\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

V: the volume of sample in sample preparation, ml

$C_{Standard}$: the concentration of standard, μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

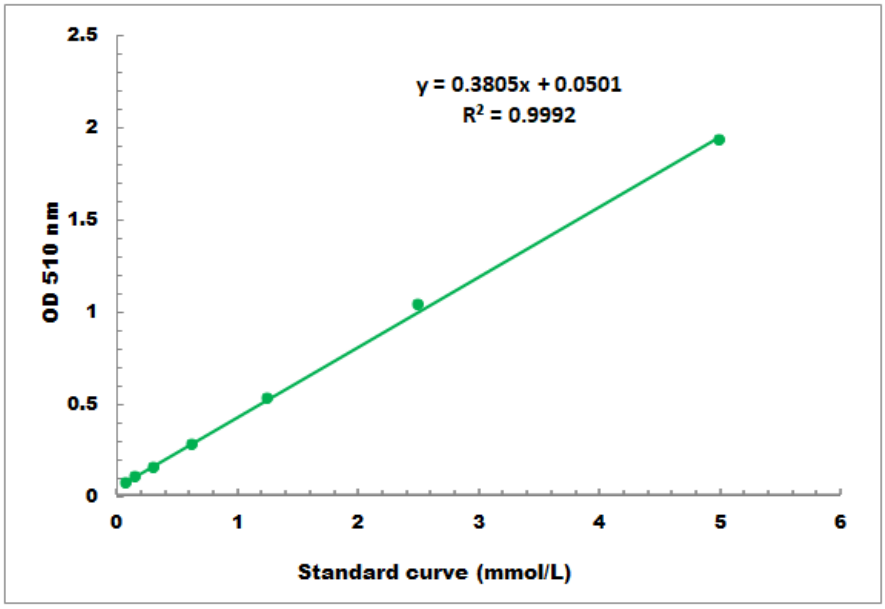
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

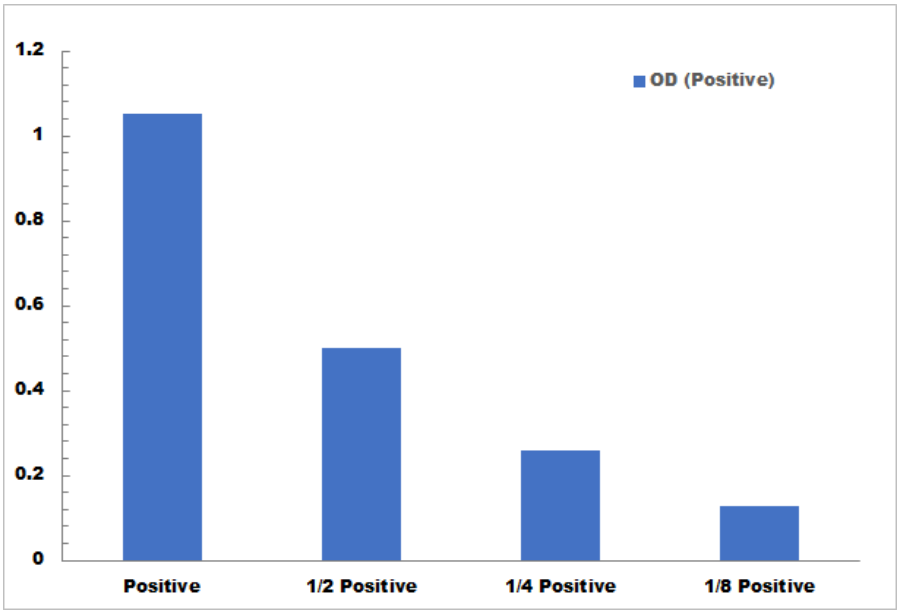
T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.04mmol/L -4mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration