Alkaline Phosphatase Activity Colorimetric Assay Kit

(Catalog #K412-500; 500 Reactions; Store kit at -20°C)

I. Introduction:

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in alkaline buffer and produces an organic radical and inorganic phosphate. Changes in alkaline phosphatase level and activity are associated with various disease states in the liver and bone. **BioVision's Alkaline Phosphatase Assay Kit** is a highly sensitive, simple, direct and HTS-ready colorimetric assay designed to measure ALP activity in serum and biological samples. It contains 10 substrate tablets providing convenience for multiple usages. The kit uses *p*-nitrophenyl phosphate (*p*NPP) as a phosphatase substrate which turns yellow (λ_{max}= 405 nm) when dephosphorylated by ALP. The Kit can detect 10-250 µU ALP in samples.

II. Kit Contents:

Components	K412-500	Cap Code	Part No.
ALP Assay Buffer	100 ml	NM	K412-500-1
*p*NPP (10 TAB)	1 vial	Red	K412-500-2
ALP Enzyme	1 vial	White	K412-500-3
Stop Solution	10 ml	WM	K412-500-4

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature (RT) before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

***p*NPP Solution:** Dissolve 2 tablets *p*NPP into 5.4 ml Assay Buffer to make 5 mM work solution. Two tablets are sufficient for 100 assays. **NEVER TOUCH THE TABLETS WITH BARE HANDS.** The *p*NPP solution is stable for 12 hours on ice.

ALP Enzyme: Reconstitute ALP Enzyme with 1 ml Assay Buffer. **DO NOT FREEZE!** The enzymes are stable for up to 2 month at 4°C after reconstitution.

Note: Ensure that the Assay Buffer is at RT before use. Keep samples, ALP Enzyme and pNPP solution on ice during the assay.

V. Alkaline Phosphatase Assay Protocol:

1. **Sample Preparations:**

Inhibitors of ALP, such as EDTA, oxalate, fluoride, and citrate should be avoided in sample preparation. Serum and plasma should be diluted 10 times; cell culture media can be measured directly. To measure intracellular ALP, washed cells (1×10^5 cells) can be homogenized in 50 µl Assay Buffer, centrifuge to remove insoluble material at 13,000g for 3 min. Add different volume of samples into 96-well plate; bring the total volume to 80 µl with Assay Buffer.

Note: Colored samples may interfere with O.D. 405 nm readings, so use a Sample Background Control. Add the same amount of sample into separate wells, bring volume to 80 µl. Add 20 µl stop solution and mix well to terminate ALP activity in the sample.

2. Add 50 µl of the 5 mM pNPP solution to each well containing the test samples and background controls. Mix well. Incubate the reaction for 60 min at 25°C, protected from light.

3. **Standard Curve Preparation:**

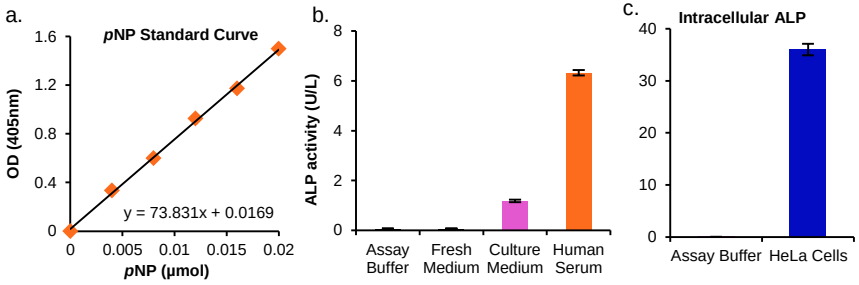
Dilute 40 µl of the 5 mM *p*NPP solution with 160 µl Assay Buffer to generate 1 mM *p*NPP standard. Add 0, 4, 8, 12, 16, 20 µl into 96-well plate in duplicate to generate 0, 4, 8, 12, 16, 20 nmol/well *p*NPP Standard. Bring the final volume to 120 µl with Assay Buffer. Add 10 µl of ALP enzyme solution to each well containing the *p*NPP standard. Mix well. The ALP enzyme will convert *p*NPP substrate to an equal amount of colored *p*-Nitrophenol (*p*NP). Incubate the reaction for 60 min at 25°C, protect from light.

4. Stop all reactions by adding 20 µl Stop Solution into each Standard and sample reaction except the Sample Background Control reaction (since 20 µl Stop Solution has been added to the Background Control when prepared in step 1), gently shake the plate. Measure O.D. at 405 nm in a micro plate reader.

5. **Calculation:** Correct background by subtracting the value derived from the 0 Standards from all Standards, Samples and Sample Background Control (The background reading can be significant and must be subtracted from sample readings). Plot *p*NP Standard Curve. Apply sample readings to the Standard Curve to get the amount of *p*NP generated by ALP sample. ALP activity of the test samples can then be calculated:

ALP activity (U/ml) = A/V/T

Where A is amount of *p*NP generated by samples (in µmol).
V is volume of sample added in the assay well (in ml).
T is reaction time (in minutes)



Figures: a. *p*NP Standard Curve. b. Measurement of ALP activity in fresh medium (80 µl, without culturing), 3-day old HeLa cell culture medium (80 µl), and human serum (80 µl, 1:10 diluted). c. Measurement of ALP activity in HeLa cells: $5X10^4$ HeLa cells were homogenized in 1 ml of Assay Buffer, diluted 1:10 in Assay Buffer and 80 µl was used to measure ALP activity. Assays were performed following the kit protocol.

VI. Unit Definition:

All the Units mentioned in this protocol are Glycine Units.

Glycine Units: The amount of enzyme causing the hydrolysis of one micromole of *p*NPP per minute at pH 9.6 and 25°C (glycine buffer).

DEA Units: The amount of enzyme causing the hydrolysis of one micromole of *p*NPP per minute at pH 9.8 and 37°C (diethanolamine buffer).

Unit Conversion: One Glycine unit as described above is equivalent to approximately three DEA units. This reaction system is in Glycine buffer.

RELATED PRODUCTS:

Acid Phosphatase Assay Kit	ADP/ATP Ratio Assay Kit
Phosphate Fluorescence Assay Kit	Phosphate Colorimetric Assay Kit
NAD/NADH Quantification Kit	NADP/NADPH Quantitation Kit
Pyruvate Assay Kit	Lactate Assay Kits
Glutamate Assay Kit	Glycogen Assay Kit
Glucose Assay Kit	Fatty Acid Assay Kit
Uric Acid Assay Kit	Sarcosine Assay Kit

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Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the substrate • Pipetting errors in the reaction mix • Air bubbles formed in well • Substrate stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		