Alkaline Phosphatase Activity Assay Kit (F)

(Catalog #K422-500; 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. The change in alkaline phosphatase level and activity is associated with a lot of diseases in the liver and bones. Alkaline phosphatase is also a popular enzyme conjugated to secondary antibody in ELISA. In **BioVision's Alkaline Phosphatase Fluorometric Assay Kit**, ALP cleaves the phosphate group of the non-fluorescent 4-Methylumbelliferyl phosphate disodium salt (MUP) substrate resulting in an intense fluorescent signal (Ex/Em = 360nm/440nm). The kit is an ultra sensitive, simple, direct and HTS-ready assay designed to measure ALP activity in serum and bio-samples with detection sensitivity ~1 µU, more sensitive than colorimetric assays. The kit is suitable for both research and drug discovery.

II. Kit Contents:

Components	K422-500	Cap Code	Part No.
ALP Assay Buffer	100 ml	NM	K422-500-1
MUP Substrate	1 vial	Red	K422-500-2
ALP Enzyme	1 vial	White	K422-500-3
Stop Solution	25 ml	WM	K422-500-4

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 prior to opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

MUP Solution: Dissolve MUP substrate into 1.2 ml Assay Buffer to generate 5 mM MUP substrate solution. The MUP solution is stable for 2 month at -20°C after dissolved.

ALP Enzyme Solution: Reconstitute ALP Enzyme with 1 ml Assay Buffer. The reconstituted enzyme is stable for up to 2 months at 4°C. **DO NOT FREEZE!**

Ensure that the Assay Buffer is at RT before use. Keep samples and ALP Solution on ice during the assay.

V. Alkaline Phosphatase Assay Protocol:

1. Sample Preparations:

Inhibitors of ALP, like tartrate, fluoride, EDTA, oxalate, and citrate, should be avoided in sample preparation. Serum, plasma, urine, semen, and cell culture media can be assayed directly. Cells (1×10^5) or tissue (~10 mg) can be homogenized in 100 µl Assay Buffer, centrifuge to remove insoluble material at 13,000g for 3 min. Add test samples directly nto 96-well plate, bring total volume to 110 µl with ALP Assay Buffer.

In order to avoid interference of components in the sample, set a sample background control. Add the same amount of samples into separate wells, bring volume to 110 µl. Add 20 µl Stop Solution and mix well to terminate ALP activity in the sample.

2. Dilute enough 5 mM MUP substrate solution to 0.5 mM with Assay Buffer (1:10); add 20 µl of the 0.5 mM MUP substrate solutions to each well containing the test samples and background controls. Mix well. Incubate the reaction for 30 min (or longer if ALP activity in sample is low) at 25°C, protected from light.

3. Standard Curve Preparation:

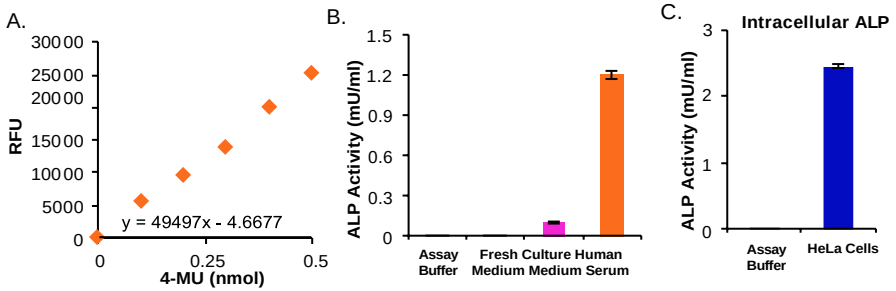
Dilute 10 µl of the 5 mM MUP solution with 990 µl ALP Assay Buffer to generate 50 µM MUP Standards. Add 0, 2, 4, 6, 8, 10 µl into 96-well plate in duplicate to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well MUP Standard. Bring the final volume to 120 µl with ALP Assay Buffer. Add 10 µl of ALP enzyme solution to each well containing the MUP Standard. Mix well. Incubate the reaction for 30 min at 25°C, protect from light. The ALP enzyme will convert MUP substrate to equal amount of fluorescent 4-Methylumbelliferone (4-MU).

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 into the background control when prepare the sample background control in step 1), gently shake the plate. Measure the fluorescence intensity at Ex/Em 360/440 nm using a fluorescence microtiter plate reader.

5. **Calculation:** Correct background by subtracting the value derived from the sample background controls for samples. Plot 4-MU standard Curve. Apply sample readings to the Standard Curve to get the amount of 4-MU generated by ALP sample. ALP activity of the test samples can be calculated:

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

Where, A is amount of 4-MU generated by samples (in nmol).
V is volume of sample added in the assay well (in ml).
T is reaction time (in minutes).



Figures: A. 4-MU Standard Curve. B. Measurement of ALP activity in fresh medium (80 µl, without culturing), 3-day old HeLa cell cultured medium (80 µl) and human serum (80 µl, 1:10 diluted). C. Measurement of ALP activity in HeLa cells: $1X10^4$ HeLa Cells were homogenized, in 200 µl of Assay Buffer, diluted 1:10 in Assay Buffer and 80 µl was used to measure intracellular ALP activity. Assays were performed according to the kit protocol.

VI. Unit Definition:

The amount of enzyme causing the hydrolysis of 1 µmol of MUP per minute at pH 10.0 and 25°C (glycine buffer).

RELATED PRODUCTS:

Alkaline Phosphatase Assay Kit
Acid Phosphatase Assay Kit
Phosphate Fluorescence Assay Kit
NAD(P)/NAD(P)H Quantification Kits
Ammonia Assay Kit
Glucose Assay Kit
Ethanol Assay Kit
ADP/ATP Ratio Assay Kit
Acid Phosphatase Fluorometric Kit
Phosphate Colorimetric Assay Kit
Lactate/Pyruvate Assay Kits
Glutamate Assay Kit
Fatty Acid Assay Kit
Uric Acid Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

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