

## Alkaline Phosphatase Fluorimetric Assay Kit

(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 Fluorimetric 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  $\mu$ U, 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	Green	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 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 diluted 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 diluted 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 room temperature 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 ( $1 \times 10^5$ ) or tissue (~10 mg) can be homogenized in 100  $\mu$ l Assay Buffer, centrifuge to remove insoluble material at 13,000g for 3 minutes. Add test samples directly into 96-well plate, bring total volume to 110  $\mu$ l with 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  $\mu$ l. Add 20  $\mu$ l 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  $\mu$ l 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, protect from light.

#### 3. Standard Curve:

Dilute 10  $\mu$ l of the 5 mM MUP solution with 990  $\mu$ l Assay Buffer to generate 50  $\mu$ M MUP standards. Add 0, 2, 4, 6, 8, 10  $\mu$ l 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  $\mu$ l with Assay Buffer.

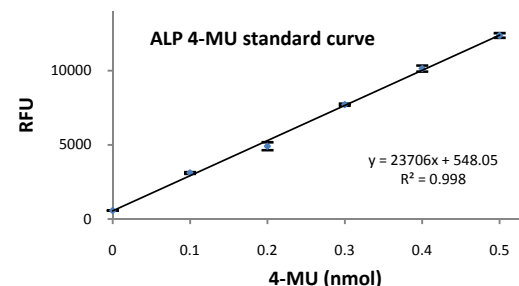
Add 10  $\mu$ l of ALP enzyme solution to each well containing the MUP standard. Mix well. Incubate the reaction for 30 min at 25°C, protect from light. The ALP enzyme will convert MUP substrate to equal amount of fluorescent 4-Methylumbelliferone (4-MU).

4. Stop all reactions by adding 20  $\mu$ l Stop Solution into each standard and sample reaction except the sample background control reaction (since 20  $\mu$ l Stop Solution has been added into the background control when prepare the sample background control in step 1), gently shake the plate. Measure 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:

$$\text{ALP activity} = A/V/T \quad (\text{mU/ml})$$

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).



**VI. Unit Definition:** The amount of enzyme causing the hydrolysis of 1  $\mu$ mol of MUP per minute at pH 10.0 and 25°C (glycine buffer).

#### VII. Related Products:

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