Alkaline Phosphatase Activity Assay Kit II

PromoKine

Instruction Manual

Catalog Number	PK-CA577-K422
Description	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 PromoKine's Alkaline Phosphatase Fluorometric Assay Kit II, 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.
Quantity	500 assays
Applications	• Measurement of alkaline phosphatase activity
Sample Type	• Tissue, cultured cells, media and biological fluids such as serum, plasma and urine

Components	Quantity	Color Code
ALP Assay Buffer	100 ml	NM
MUP Substrate	1 vial	Red
ALP Enzyme	1 vial	Green
Stop Solution	25 ml	WM

User Supplied Reagents & Equipment	• Spectrofluorometer/ Multiwell fluorescence reader • 96-well white or black plate with flat bottom
Storage and Reagents Preparation	Store kit at -20°C, protected from light. Briefly centrifuge all small vials prior to opening. Allow Assay Buffer to warm to room temperature before use. Read entire protocol before performing the assay procedure. • **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 room temperature before use. Keep samples and ALP Solution on ice during the assay.
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 minutes. Add test samples directly into 96-well plate, bring total volume to 110 µl 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 µl. Add 20 µl Stop Solution and mix well to terminate ALP activity in the sample. **2. Reaction Mix**: 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 minutes (or longer if ALP activity in sample is low) at 25°C, protect from light. **3. Standard Curve**: Dilute 10 µl of the 5 mM MUP solution with 990 µl 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 Assay Buffer. Add 10 µl of ALP enzyme solution to each well containing the MUP standard. Mix well. Incubate the reaction for 30 minutes at 25°C, protect from light. The ALP enzyme will convert MUP substrate to equal amount of fluorescent 4-Methylumbelliferone (4-MU). **4. Measurement**: 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 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).
Intended Use	For in vitro research use only. Not for diagnostic or therapeutic procedures.

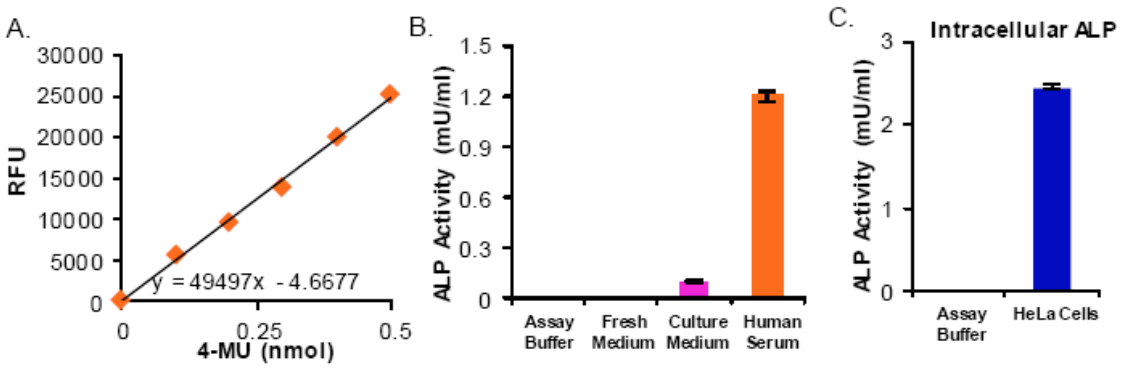

Figure: A. 4-MU Standard Curve. B. Measurement of ALP activity in fresh medium (80 µl, without culturing), 3-day old HeLa cell cultured medium (80 µl) and human serum (80 µl, 1:10 diluted). C. Measurement of ALP activity in HeLa cells: $1X10^4$ HeLa Cells were homogenized, in 200 µl of Assay Buffer,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

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