Product Information

Alkaline Phosphatase Activity Fluorometric Assay Kit

I. Kit Contents:

Components	K2077-500 500 assays	Cap Color	Part Number
ALP Assay Buffer	100 ml	NM	K2077-C-1
MUP Substrate	1 vial	Red	K2077-C-2
ALP Enzyme	1 vial	Green	K2077-C-3
Stop Solution	25 ml	WM	K2077-C-4

II. Introduction:

Alkaline phosphatase (ALP) is a hydrolase enzyme that removes phosphate groups from nucleotides proteins and alkaloids. Changes in ALP level and activity are associated with various disease states in the bone and liver. ALP is also a common enzyme conjugated to secondary antibody used in ELISA.

The Alkaline Phosphatase Activity Fluorometric Assay Kit provides a highly sensitive and convenient way for detection of ALP activity based on fluorometric method in serum and biological samples. In the assay, ALP removes the phosphate group of the non-fluorescent 4-Methylumbelliferyl phosphate disodium salt (MUP) substrate producing an intense fluorescent signal (Ex/Em = 360 nm/440 nm). The kit is an ultra sensitive, HTS-ready assay that is more sensitive than colorimetric method with detection sensitivity ~1 μU. The assay is suited for both research and drug discovery.

III. Storage and Handling:

Store the kit at -20℃, 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 Assay Buffer to generate 5 mM MUP substrate solution. The MUP solution is stable for 2 month at -20℃ 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℃. 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 (1×10^5) or tissue (~ 10 mg) can be homogenized in 100 μl Assay Buffer, centrifuge to remove insoluble material at 13,000 g 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. 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℃, 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 min at 25℃, 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 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).

Unit Definition: The amount of enzyme causing the hydrolysis of 1 µmol of MUP per minute at pH 10.0 and 25℃ (glycine buffer).

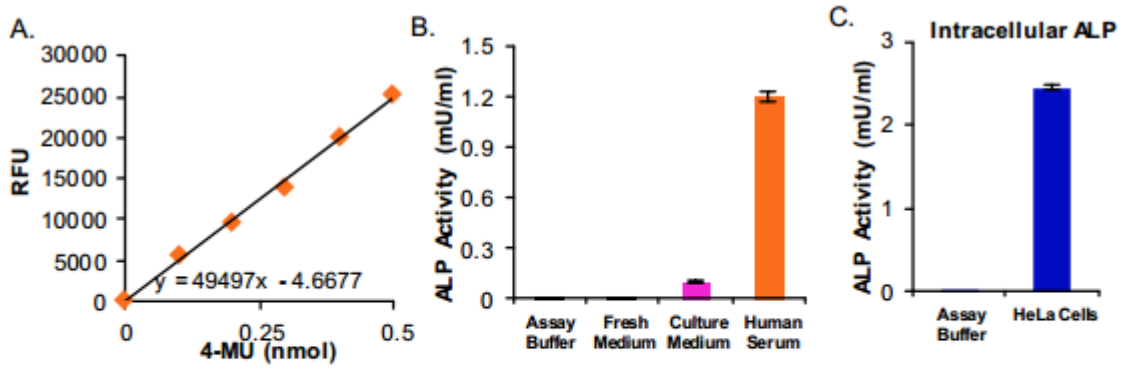

Figure: A. 4-MU Standard Curve. B. Measurement of ALP activity in fresh medium (80 µl, without culturing), 3-day old HeLa cell cultured medium (80 µl) and human serum (80 µl, 1:10 diluted). C. Measurement of ALP activity in HeLa cells: $1X10^4$ HeLa Cells were homogenized, in 200 µl of Assay Buffer,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

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different 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 prepared in a different buffer	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet

	• Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • 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 data sheet & 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 standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard 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.		

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Our promise

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