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Product Information

Acid Phosphatase Activity Fluorometric Assay Kit

Catalog Number **MAK087**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Acid phosphatases (AP) are a family of enzymes that non-specifically catalyze the hydrolysis of monoesters and anhydrides of phosphoric acid to produce inorganic phosphate at an optimal pH of 4–7. Changes in the levels and activities of AP occur in many patho-physiological conditions including prostrate cancer and bone disease.

The AP Activity Fluorometric Assay Kit provides a simple and direct procedure for measuring AP activity in serum and other samples. AP activity is determined by the conversion of the non-fluorescent 4-methyl-umbelliferyl phosphate disodium salt (MUP) to the fluorescent product 4-methylumbelliferone (4-MU) (λ_{ex} = 360/λ_{em} = 440 nm), proportional to the AP activity present. One unit of AP is the amount of enzyme that will hydrolyze 1.0 μmole of MUP per minute at 25 °C.

Components

The kit is sufficient for 500 assays in 96 well plates.

AP Assay Buffer Catalog Number MAK087A	100 mL
MUP Substrate Catalog Number MAK087B	1 vl
AP Enzyme Catalog Number MAK087C	1 vl
Stop Solution Catalog Number MAK087D	25 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

AP Assay Buffer – Allow buffer to come to room temperature before use.

MUP Substrate – Reconstitute with 1.2 mL of AP Assay Buffer to generate a 5 mM MUP substrate solution. Mix well by pipetting (don't vortex), then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

AP Enzyme – Reconstitute with 1 mL of AP Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at 4 °C. Do not freeze enzyme solution and use within 2 months of reconstitution. Keep enzyme cold while in use.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, unless otherwise specified, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

MUP Standards for Fluorometric Detection
Dilute 10 µL of the 5 mM MUP substrate solution with 990 µl of AP Assay Buffer to prepare a 50 µM MUP solution. Add 0, 2, 4, 6, 8, and 10 µL of the 50 µM MUP solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add AP Assay Buffer to each well to bring the volume to 120 µL.

Sample Preparation
During sample preparation, avoid inhibitors of AP including tartrate, fluoride, EDTA, oxalate, and citrate.

Liquid samples such as serum, plasma, urine, semen, and cell culture media can be assayed directly.

Tissue (10 mg) or cells (1×10^5) can be homogenized in 100 µL of ice-cold AP Assay Buffer. Centrifuge the samples at 13,000 × *g* for 5 minutes to remove insoluble material.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

To control for background interference, set a sample blank for each test sample.

Bring all samples (test and blank) to a final volume of 110 µL with AP Assay Buffer. Add 20 µL of Stop Solution to the sample blank wells and mix well to terminate AP activity.

Assay Reaction
1. For the samples and sample blanks, prepare a 10-fold dilution of the 5 mM MUP substrate solution to prepare a 0.5 mM MUP substrate solution. Add 20 µL to each of the test sample and sample blank wells.

2. Add 10 µL of the AP Enzyme solution to each of the MUP standard wells to convert the MUP to 4-MU.

3. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30–60 minutes at room temperature. Cover the plate and protect from light during the incubation.

4. Add 20 µL of the Stop Solution to each of the test sample and standard wells but not the sample blank wells. Mix well using a horizontal shaker or by pipetting.

5. Measure the fluorescence intensity (λ_{ex} = 360/ λ_{em} = 440 nm).

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) MUP standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate standards to plot a 4-MU standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the sample blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of 4-MU present in the samples may be determined from the standard curve.

$$\text{AP Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of 4-MU generated by samples
Reaction Time = in minutes
V = sample volume (mL) added to well

AP activity is reported as μmole/min/mL. One unit of AP is the amount of enzyme that will hydrolyze 1.0 μmole of MUP per minute at 25 °C.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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