ab83370

Acid Phosphatase Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Acid Phosphatase in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 3a, Last Updated 14 June 2023

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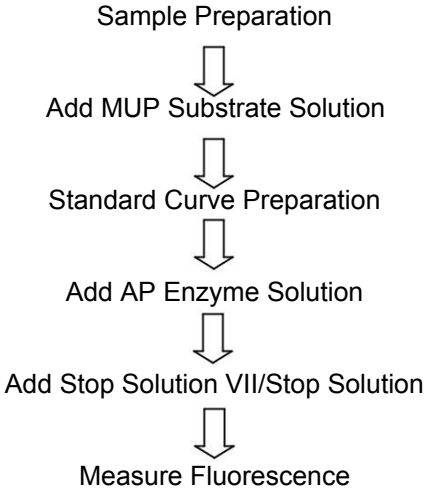
1. Overview

Acid phosphatase (AP) dephosphorylates phosphate groups from the phosphate esters in acid conditions. Different forms of acid phosphatase are found in different organs, and their serum levels are used as a diagnosis for disease in the corresponding organs. For example, elevated prostatic acid phosphatase levels may indicate the presence of prostate cancer and elevated tartrate resistant acid phosphatase levels may indicate the bone disease.

Abcam's Acid Phosphatase Assay Kit (Fluorometric) uses non-fluorescent Methylumbelliferyl phosphate disodium (MUP) as the substrate which has Ex/Em=360/440 nm when dephosphorylated by AP. The kit is an ultra-sensitive, simple, direct and HTS-ready fluorometric assay designed to measure AP activity in serum and other bio-samples. The detection sensitivity is ~1 µU, more sensitive than colorimetric assays. The kit is suitable for both research and drug discovery.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
AP Assay Buffer	100 mL
MUP Substrate	1 vial
AP Enzyme	1 vial
Stop Solution VII/Stop Solution	25 mL

* 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.
- Keep samples and AP Enzyme Solution on ice during the assay.
- Read the entire protocol before performing the assay.

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.

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

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

a. **For cells and tissue:** Cells (1×10^5) or tissue (~10 mg) can be homogenized in 100 µl Assay Buffer, centrifuge to remove insoluble material at 13,000 x g for 3 minutes.

b. **Serum, plasma, urine, semen, and cell culture media** can be assayed directly.

Add test samples directly into a 96-well plate, bringing total volume to 110 µl with Assay Buffer.

Notes:

a) Inhibitors of AP, like tartrate, fluoride, EDTA, oxalate, and citrate, should be avoided in sample preparation.

b) 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 VII/Stop Solution and mix well to terminate AP 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 AP activity in sample is low) at 25°C, protect from light.

3. Standard Curve Preparation:

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

4. Stop all reactions by adding 20 µl Stop Solution VII/Stop Solution into each standard and sample reaction except the sample background control reaction (since 20 µl Stop Solution VII/Stop Solution has been added into the background control when preparing the sample background control in step 1), gently shake the plate.

5. Measure fluorescence intensity at Ex/Em 360/440 nm using a fluorescence microtiter plate reader.

5. Data Analysis

Correct background by subtracting the value derived from the sample background controls for samples.

Plot the 4-MU standard Curve. Apply sample readings to the standard curve to get the amount of 4-MU generated by AP sample.

AP activity of the test samples can be calculated:

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

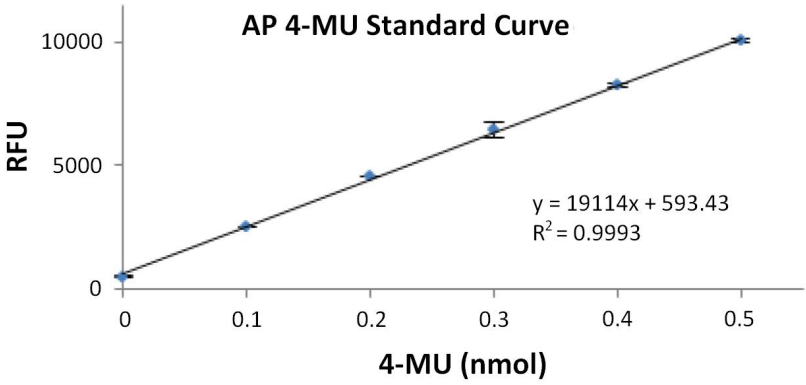
Where:

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 25°C.


AP 4-MU Standard Curve
RFU
10000
5000
0
0
0.1
0.2
0.3
0.4
0.5
4-MU (nmol)
y = 19114x + 593.43
R2 = 0.9993

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms) Luminescence: White plates Colorimetry: Clear plates If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:

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