

Acid Phosphatase (ACP) Assay Kit (Fluorometric)

LS-K223-100 (100 Tests) • Store at -20°C



Introduction

Acid Phosphatase (ACP) is an enzyme which catalyzes the cleavage of phosphate groups from other molecules during digestion. Acid phosphatase can be found in lysosomes and become active after fusing with endosomes, acidifying the pH and, thus, creating an optimal environment for ACP. ACP can also be found in bone, spleen, liver, kidney and blood. Serum levels can be used as a biomarker for prostatic carcinoma, although prostate-specific antigen (PSA) is more widely used. This non-radioactive, fluorometric ACP assay is based on the cleavage of methylumbelliferyl phosphate. Methylumbelliferone becomes intensely fluorescent after addition of the stop reagent. The increase in fluorescence at 360/450 nm after addition of the stop reagent is directly proportional to the enzyme activity.

Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 0.008 to 10 U/L for a 30 minute reaction.
- High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- Acid Phosphatase activity determination in biological samples (e.g. plasma, serum, cell lysate, tissue samples.)

Components

Component	K223-100
	100 Tests
Assay Buffer	12 mL
MUP Substrate	120 µL
Stop Reagent	12 mL
Standard	120 µL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor), black flat-bottom 96-well plates (e.g. VWR cat# 89089-582), centrifuge tubes and plate reader.

Storage

The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

FOR RESEARCH USE ONLY! Not for use in humans.

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Assay Procedure

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and Stop Reagent to samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Sample Preparation

Serum and plasma should be diluted 2-5 fold.

Tissue: Prior to dissection, rinse tissue in Tris buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 μ L 50 mM Tris buffer (pH 7.5). Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold 50 mM Tris buffer (pH 7.5), approximately one million cells per mL. Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

All samples can be stored at -80 to -20°C for at least one month.

Reagent Preparation

Equilibrate all components to desired reaction temperature (e.g. 25°C or 37°C).

Standard Preparation

Mix 25 μ L of Standard (MUB) with 475 μ L dH₂O to make 100 μ M Premix.

No	Premix + dH ₂ O	Vol (μ L)	MUB (μ M)
1	100 μ L + 0 μ L	100	100
2	60 μ L + 40 μ L	100	60
3	30 μ L + 70 μ L	100	30
4	0 μ L + 100 μ L	100	0

Procedure

1. Transfer 20 μ L of each sample into separate wells. Transfer 20 μ L of each standard into wells of a black flat-bottom 96-well plate.
2. The Working Reagent is prepared by mixing together for each well 85 μ L of assay buffer and 1 μ L of MUP Substrate. Add 80 μ L of Working Reagent to all standard and sample wells. Tap plate briefly to mix.
3. Incubate at 25°C or desired temperature for 30 minutes. Add 50 μ L of Stop Reagent to each well. Tap plate briefly to mix.
4. Read Fluorescence at 360/450nm.

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Calculations

Subtract blank F (water, #4) from the standard F values and plot the ΔF against standard concentrations. Determine the Slope and use the following equation to calculate Acid Phosphatase activity.

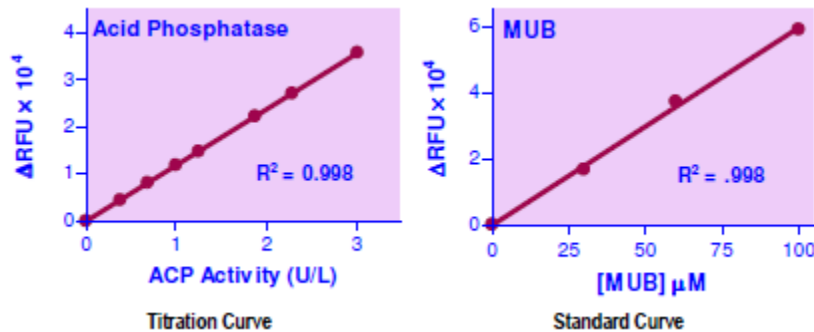
$$\text{ACP Activity} = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{\text{Time} \cdot \text{Slope}} \times n \quad (\text{U/L})$$

where F_{SAMPLE} is the RFU value for each sample and F_{BLANK} is the RFU value of the water (standard #4). Slope is the slope of the linear regression fit of the standard points and Time is the reaction time (30 min). n is the dilution factor.

Unit definition: 1 Unit (U) of ACP will catalyze the conversion of 1 μmole of 4-methylumbelliferyl phosphate to 4-methylumbelliferone and phosphate per min at 25°C and pH 5.3.

Note: If sample ACP activity exceeds 10 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with ACP activity < 0.1 U/L, the incubation time can be extended up to 60 minutes for greater sensitivity.

Sample Data



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