Technical Bulletin

Acid Phosphatase Fluorometric Assay Kit

Catalogue number MAK525

Product Description

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.

The Acid Phosphatase Fluorometric Assay Kit is a non-radioactive, fluorometric ACP assay is based on the cleavage of a synthetic substrate. The product methylumbelliferone (MUB) becomes intensely fluorescent after addition of the stop reagent. The increase in fluorescence (λ_{Ex} = 360 nm/λ_{Em} = 450 nm) after addition of the stop reagent is directly proportional to the enzyme activity.

The linear detection range of the kit is (20 µL sample): 0.008 to 10 U/L for a 30-minute reaction. The kit is used to determine Acid Phosphatase activity in biological samples, such as plasma, serum, cell lysate, tissue samples.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer 12 mL
  Catalogue Number MAK525A
- MUP Substrate 120 µL
  Catalogue Number MAK525B
- Stop Reagent 12 mL
  Catalogue Number MAK525C
- MUB Standard 120 µL
  Catalogue Number MAK525D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescent multiwell plate reader
- Black flat-bottom 96-well or 384-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set
  (Catalogue Number D9063 or equivalent).

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Note: 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:

1. Prior to dissection, rinse tissue in Tris buffered saline (pH 7.4) to remove blood.
2. Homogenize tissue (50 mg) in ~ 200 µL 50 mM Tris buffer (pH 7.5).
3. Centrifuge at 14,000 x g for 10 minutes at 4 °C.
4. Remove supernatant for assay.

Cell Lysate:

1. Collect cells by centrifugation at 2,000 x g for 5 minutes at 4 °C.

Note: For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman.

2. Homogenize or sonicate cells in an appropriate volume of cold 50 mM Tris buffer (pH 7.5), approximately one million cells per mL.
3. Centrifuge at 14,000 x g for 10 minutes at 4 °C.
4. Remove supernatant for assay.

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

Procedure

All Samples and Standards should be run in duplicate.
Note: This procedure is written for 96-well plates.

Standard Curve Preparation

1. Prepare 100 µM standard by mixing 25 µL of Standard (MUB) with 475 µL purified water.
2. Dilute the 100 µM standard according to Table 1.

Table 1

Well No.	Standard (µL)	Purified water (µL)	MUB Conc. (µM)
1	100	0	100
2	60	40	60
3	30	70	30
4	0	100	0

Reaction Preparation

1. Transfer 20 µL of each sample into separate wells.
2. Transfer 20 µL of each standard into wells of a black flat-bottom 96-well plate.

Working Reagent Preparation

Mix enough reagents for the number of assays to be performed. For each Standard and Sample well, prepare 80 µL of Working Reagent according to Table 2.

Table 2.

Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	85 µL
MUP Substrate	1 µL

1. Add 80 µL of Working Reagent to all Standard and Sample wells.
2. Tap plate briefly to mix.

Measurement

1. Incubate at 25 °C or desired temperature for 30 minutes.
2. Add 50 µL of Stop Reagent to each well. Tap plate briefly to mix.
3. Read fluorescence (F) at λ_{Ex} =360 nm/λ_{Em} =450 nm.

Results

1. Subtract blank fluorescence (F) (standard #4) from the standard fluorescence values and plot the ΔF against standard concentrations.
2. Determine the slope.
3. Use following equation to calculate Acid Phosphatase Activity.

Acid Phosphatase Activity (U/L) =

$$\frac{F_{Sample} - F_{Blank}}{\text{Time x Slope}} \times DF$$

Where:

F_{SAMPLE} = RFU value for each sample
F_{BLANK} = RFU value for water (standard 4)
Slope = slope of the linear regression fit of the standard points
Time = reaction time (30 min)
DF = 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.

Figure 1.
Typical Titration curve

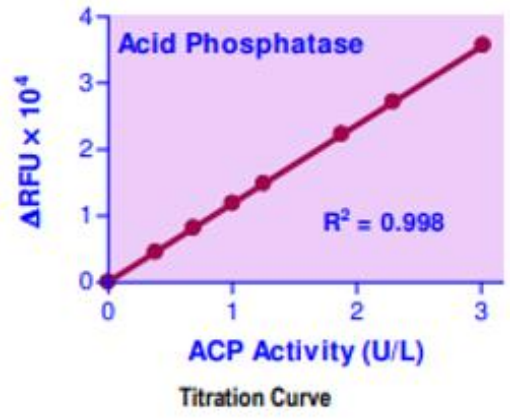
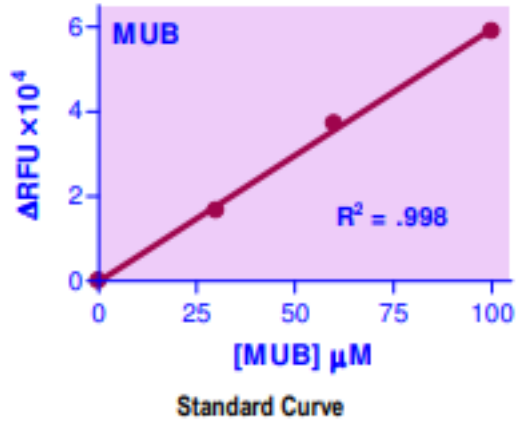


Figure 2.
Typical Standard Curve



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