Technical Bulletin

Acid Phosphatase Assay Kit

Catalog Number MAK446

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 becomes active after fusing with endosomes, acidifying the pH and 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.

The non-radioactive, colorimetric Acid Phosphatase Assay Kit is based on the cleavage of *p*-nitrophenol from the synthetic substrate. *p*-Nitrophenol becomes intensely colored after addition of the stop reagent. The increase in absorbance at 405 nm after addition of the stop reagent is directly proportional to the enzyme activity. The assay method has a linear detection range of 0.05 to 60 U/L for a 30 minute reaction and a sample size of 20 µL.

The kit is suitable for the determination of acid phosphatase in biological samples (e.g., plasma, serum, cell lysate, tissue samples.)

Components

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

- Assay Buffer 12 mL
  Catalog Number MAK446A
- pNPP Liquid 280 µL
  Catalog Number MAK446B
- Stop Reagent 12 mL
  Catalog Number MAK446C
- Standard (12.5 mM) 1 mL
  Catalog Number MAK446D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥ 14,000 × *g*
- Tris Buffered Saline (TBS) (Catalog Number T5912 or equivalent)
- Trizma® Base Solution (Catalog Number T1699 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 on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to desired reaction temperature (25 °C or 37 °C) prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum and plasma

Serum and plasma should be diluted 2-5 fold.

Tissue

1. Prior to dissection, rinse tissue in TBS, pH 7.4, to remove blood.
2. Homogenize tissue (50 mg) in 200 µL of 50 mM Tris buffer, pH 7.5.
3. Centrifuge homogenized tissue at 14,000 × *g* for 10 minutes at 4 °C.
4. Remove supernatant and retain for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × *g* for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold 50 mM Tris buffer, pH 7.5. Use approximately one million cells per mL of buffer.
4. Centrifuge at 14,000 × *g* for 10 minutes at 4 °C.
5. Remove supernatant and retain for assay.

All Samples

1. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
2. All samples can be stored at –20 °C to -80 °C for at least one month.
3. Transfer 20 µL of each Sample to separate wells of a clear 96-well plate.
4. For visually colored or opaque samples, prepare a Sample Blank by transferring 20 µL of the Sample into parallel wells of the plate. Add 80 µL of purified water to the Sample Blank well.

Standard Curve Preparation

1. Prepare a 1000 µM Nitrophenol Standard by mixing 20 µL of the 12.5 mM Nitrophenol Standard with 230 µL of purified water.
2. Prepare Nitrophenol Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Nitrophenol Standards

Well	1000 µM Nitrophenol Standard	Purified Water	Nitrophenol (µM)
1	100 µL	-	1000
2	60 µL	40 µL	600
3	30 µL	70 µL	300
4	-	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96-well plate.

Working Reagent

Mix enough reagents for the number of assays to be performed. For each Standard and Standard well, prepare 87 µL of Working Reagent according to Table 2.

Table 2.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	85 µL
pNPP Liquid	2 µL

Measurement

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 multichannel pipettor is recommended.

1. Add 80 µL of Working Reagent to all Standard and Sample wells. Do not add Working Reagent to Sample Blank wells. Tap plate briefly to mix.
2. Incubate plate at 25 °C or desired temperature for 30 minutes.
3. Add 50 µL of Stop Reagent to each well. Tap plate briefly to mix.
4. Read optical density (OD) at 405 nm.

Results

1. Subtract the OD_{Blank} (Standard #4) from the remaining Standard OD readings. Plot the corrected Standard OD readings against the Standard concentrations.
2. Determine the slope of the Standard curve using linear regression.
3. Calculate the Acid Phosphatase activity of the sample:

   Acid Phosphatase (U/L) =

$$\frac{OD_{Sample} - OD_{Blank}}{Time \times Slope} \times DF$$

where

OD_{Sample} = OD value at 405 nm of Sample

OD_{Blank} = OD value at 405 nm of Blank (Standard #4)

Time = Reaction time (30 minutes)

Slope = Slope of the Standard curve

DF = Sample Dilution Factor (DF = 1 for undiluted Samples)

If the sample ACP activity exceeds 60 U/L, repeat the assay and either use a shorter reaction time or dilute samples in purified water. For samples with ACP activity <1 U/L, the incubation time can be extended up to 60 minutes for greater sensitivity.

Unit definition: 1 Unit (U) of Acid Phosphatase will catalyze the conversion of 1 µmole of p-Nitrophenyl phosphate to p-Nitrophenol and phosphate per minute at 25 °C and pH 5.3.

Figure 1.
Typical Nitrophenol Standard Curve

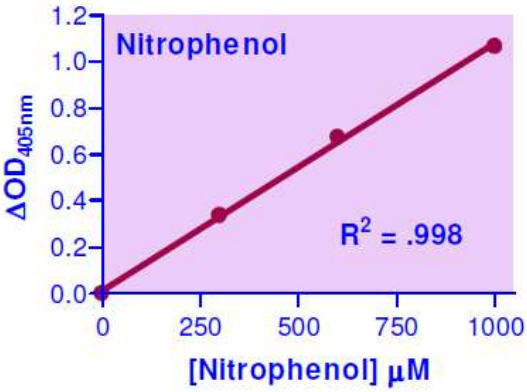
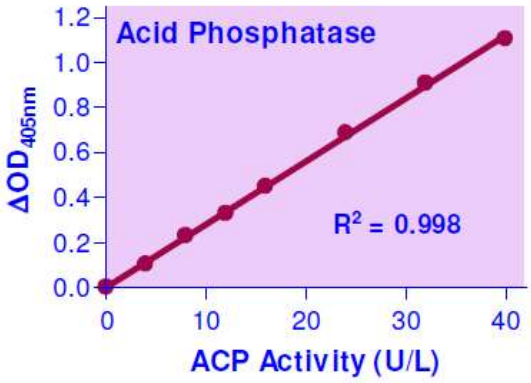


Figure 2.
Typical Titration Curve



References

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2. Bull, H., et al., Acid Phosphatases. *Mol. Pathol.,* **55(2)**, 65–72 (2002).
3. Taira, A., et al., Reviving the acid phosphatase test for prostate cancer. *Oncology (Williston Park, N.Y.),* **21(8)**, 1003–10 (2007).

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