# Acid Phosphatase Colorimetric Assay Kit

Item No. 10008051

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# TABLE OF CONTENTS



# GENERAL INFORMATION

## Materials Supplied

| Item Number | Item | Quantity/Size |
|---|---|---|
| 10009146 | Acid Phosphatase Assay Buffer (10X) | 1 vial/10 ml |
| 10009147 | Acid Phosphatase Substrate | 1 vial/16 tablets |
| 10009148 | Acid Phosphatase Stop Solution | 1 vial/25 ml |
| 10009149 | Acid Phosphatase (control) | 5 vials/lyophilized |
| 10009150 | Acid Phostphatase Sodium Tartrate | 1 vial/5 ml |
| 400014 | 96-Well Solid Plate (Colorimetric Assay) | 5 plates |
| 400012 | 96-Well Cover Sheet | 5 covers |

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

It is recommended to take appropriate precautions when using the kit reagents (*i.e.*, lab coat, gloves, eye goggles, etc.) as some of them may be harmful.

The sodium hydroxide solution is corrosive and harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

## If You Have Problems

**Technical Service Contact Information**

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**E-Mail:** techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored at 4°C and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader capable of measuring an absorbance of 405-415 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable.



# INTRODUCTION

## Background

Acid phosphatases (APs) are members of the hydrolase class of enzymes and can be found in both plant and animal species. They are grouped together because of the shared ability to catalyze the hydrolysis of orthophosphate monoesters under acidic conditions.[1] Despite having a common functional identity, AP isoenzymes differ widely regarding tissue and chromosomal origin, molecular weight, amino acid homology, sequence length, and resistance to L-tartrate or to fluoride (see Table 1).[1]

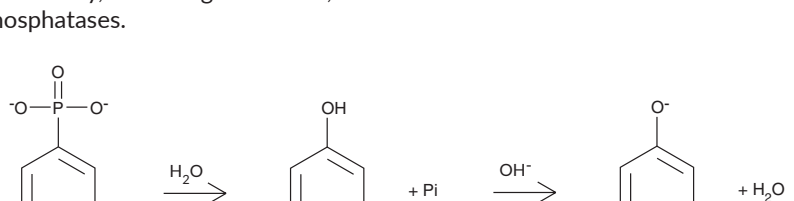
| Acid Phosphatase | Tissue/Cells of Origin | MW (kDa) | Tartrate Resistant | Fluoride Resistant |
|---|---|---|---|---|
| Lysosomal | Most cells | 100 | - | + |
| Prostatic | Prostate gland, brain, liver, spleen, platelets | 100 | - | + |
| Erythrocytic | Erythrocytes, Many cell types | 18 | + | - |
| Macrophagic | Macrophages of liver, spleen, lung | 37 | + | - |
| Osteoclastic | Osteoclasts of bone | 37 | + | - |

**Table 1.**

Human acid phosphatases are normally found in low concentrations. However, pronounced changes in enzyme synthesis occur in particular diseases, resulting in unusually high or low concentrations. Thus, AP levels are often used as clinical markers of disease. The levels of prostate acid phosphatase (PAP) have long been used as an indicator of prostate cancer, while an increased level of tartrate resistant acid phosphatase (TRAP) is often indicative of bone disease.[1,2]

## About This Assay

Cayman's Acid Phosphatase Colorimetric Assay Kit provides a convenient method for detecting total AP activity in plasma, serum, urine, and semen. The assay utilizes *para*-nitrophenyl phosphate (*p*NPP) as a chromogenic substrate for the enzyme. In the first step, AP dephosphorylates *p*NPP. In the second step, the phenolic OH-group is deprotonated under alkaline conditions resulting in *p*-nitrophenolate that yields an intense yellow color which can be measured at 405-414 nm (see scheme).[3] The kit provides all reagents needed to assay AP activity, including L-tartrate, an inhibitor of non-tartrate resistant acid phosphatases.




*p*NPP *p*-nitrophenole *p*-nitrophenolate

**Figure 1. Scheme**

# PRE-ASSAY PREPARATION

## Reagent Preparation

1. **Acid Phosphatase Assay Buffer (10X) - (Item No. 10009146)**

   Dilute 5 ml of Assay Buffer with 45 ml of HPLC-grade water. This final Assay Buffer (100 mM HEPES, pH 5.0) should be used for the dilution of samples and dissolving the Acid Phosphatase Substrate. The diluted Assay Buffer is stable for at least one month if stored at room temperature.

2. **Acid Phosphatase Substrate - (Item No. 10009147)**

   The vial contains *p*-nitrophenylphosphate (*p*NPP) tablets. Dissolve two tablets in 3 ml of diluted Assay Buffer. Two tablets are sufficient to assay one 96-well plate. *CAUTION: To prevent contaminating the tablets, avoid touching the tablets with bare hands.* The *p*NPP solution is stable for four hours.

3. **Acid Phosphatase Stop Solution - (Item No. 10009148)**

   The vial contains a solution of 2 M sodium hydroxide. Dilute 15 ml of this solution with 45 ml of HPLC-grade water for a final concentration of 500 mM. The diluted Stop Solution is stable for at least one month if stored at room temperature.

4. **Acid Phosphatase (control) - (Item No. 10009149)**

   The vial contains a lyophilized powder of wheat germ acid phosphatase (AP). Dissolve the powder with 2 ml of diluted Assay Buffer and store on ice. A 20 µl aliquot of the enzyme should produce an $A_{405}$ ≥0.5 in the assay. The resuspended enzyme should be used within one hour.

5. **Acid Phosphatase Sodium Tartrate - (Item No. 10009150)**

   This vial contains a solution of sodium tartrate and its use is optional. It can be used to inhibit non-tartrate resistant acid phosphatases, such as prostatic and lysosomal acid phosphatases. The solution is ready to use as supplied.

## Sample Preparation

### Plasma

Typically, human plasma has a total acid phosphatase level of 2-7.9 U/liter.[4]

1. Collect blood using an anticoagulant such as heparin or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80°C. The plasma sample will be stable for one month. Repeated freeze/thaw cycles are not encouraged, as the activity greatly decreases.
3. Plasma does not need to be diluted before assaying. When assaying plasma for AP activity, we recommend running a plasma blank so that the background absorbance can be subtracted from the plasma sample.

### Serum

Typically, human serum has a total acid phosphatase level of 2.5-11.7 U/liter.[5]

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The sample will be stable for one month. Repeated freeze/thaw cycles are not encouraged, as the activity greatly decreases.
4. Serum does not need to be diluted before assaying. When assaying serum for AP activity, we recommend running a serum blank so that the background absorbance can be subtracted from the serum sample.

### Urine

Urine does not require any special treatment, other than potential dilution with diluted Assay Buffer. If not assaying the same day, freeze at -80°C.

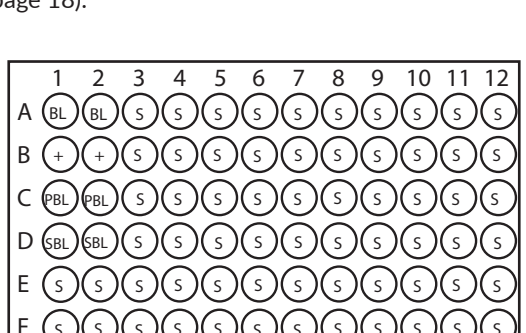
### Semen

Semen contains very high concentrations of acid phosphatase, ranging from 87 to 436 KU/liter.[6] It will require significant dilution (*i.e.*, 1:2,000 to 1:4,000) to fall within parameters of the assay. Semen can be diluted with Assay Buffer. If not assaying the same day, freeze at -80°C.

# ASSAY PROTOCOL

## Plate Set Up

There is no specific pattern for using the wells on the plate. We suggest that there be at least two wells designated as positive controls. A typical layout of blanks, positive controls, and samples to be measured in duplicate is given (see Figure 2 below). We suggest you record the contents of each well on the template sheet provided (see page 18).




BL = Blank Wells
\+ = Positive Control Wells
PBL = Plasma Blank Wells
SBL = Serum Blank Wells
S = Sample Wells

**Figure 2. Sample plate format**

### Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps to maintain more precise times of incubation. Use different tips to pipette enzyme, AP substrate, and assay buffer..
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

**General Information**

- The final volume of the assay is 150 µl in all the wells.
- All reagents except samples must be equilibrated to room temperature before beginning the assay.
- The assay temperature is 37°C.
- It is not necessary to use all the wells on the plate at one time.
- If the activity of the sample is not known or if it is expected to be beyond the range of the assay, it is prudent to assay the sample at several dilutions.
- It is recommended that the samples and controls be assayed at least in duplicate.
- When assaying plasma or serum, it is recommended that plasma or serum blanks be also assayed.
- 30 samples can be assayed in triplicate or 46 in duplicate.

## Performing the Assay

1. **Blank Wells** - add 30 µl of Assay Buffer to two wells.
2. **Plasma or Serum Blank Wells** - add 10 µl of Assay Buffer and 20 µl of either plasma or serum to two wells per sample.
3. **Positive Control Wells (Acid Phosphatase)** - add 10 µl of Assay Buffer and 20 µl of Acid Phosphatase (control) to at least two wells.
4. **Samples Wells** - add 10 µl of Assay Buffer and 20 µl of sample to each well being used. If measuring non-tartrate-resistant acid phosphatase, replace the 10 µl of Assay Buffer with 10 µl of Acid Phosphatase Sodium Tartrate (Item No. 10009150). *NOTE: By assaying the sample with and without the inhibitor, you will measure the tartrate resistant (erythrocytic, macrophagic, and osteoclastic) AP and the total AP activities, respectively. Subtracting the tartrate resistant value from the total AP value will give you the non-tartrate resistant (lysosomal and prostatic) AP activity. To obtain reproducible results, sample AP levels should fall within 0-0.05 U/ml or OD range of 0-1.5. When necessary, samples can be diluted with Assay Buffer to bring the AP activity to this level.*
5. Initiate the reaction by adding 20 µl of AP Substrate Solution to each well being assayed *except* plasma or serum blank wells.
6. Cover the plate with the plate cover and incubate for 20 minutes at 37°C.
7. Remove the plate cover and add 100 µl of diluted Stop Solution to each well.
8. Add 10 µl of AP Substrate Solution to the plasma and serum blank wells.
9. Read the absorbance at 405-414 nm using a plate reader.



# ANALYSIS

## Calculations

1. Calculate the average absorbance of the blanks, positive control, and each sample.
2. Subtract the average absorbance of the blank from all samples and the positive control. This is the adjusted absorbance used in the equation below. Use plasma or serum blanks for correcting plasma and serum samples.
3. Calculate the acid phosphatase activity of the samples using the following equation. One unit is the amount of the acid phosphatase required to release 1 µmol of phosphate from *p*NPP in one minute at 37°C.

**AP Activity (µmol/min/ml) =**

$$\frac{\Delta A_{405}}{[20\ (\text{min.}) \times (*10.68\ \text{mM}^{-1})]} \times \frac{0.15\ \text{ml}}{0.02\ \text{ml}} \times \text{Sample dilution}$$

*The actual extinction coefficient for *p*NPP is 17.8 $\text{mM}^{-1}\text{cm}^{-1}$. The value has been adjusted for the pathlength of the solution in the well (0.6 cm).

**Optional**

**Non-Tartrate resistant AP Activity (µmol/min/ml) =**

$$\frac{\Delta A\ (\text{without inhibitor}) - \Delta A\ (\text{with inhibitor})}{[20\ (\text{min.}) \times (*10.68\ \text{mM}^{-1})]} \times \frac{0.15\ \text{ml}}{0.02\ \text{ml}} \times \text{Sample dilution}$$

## Performance Characteristics

**Precision:**

When a series of 86 human urine measurements were performed on the same day under the same experimental condition, the intra-assay coefficient of variation was 1.27%. When a series of eight human urine measurements were performed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 2.26%.

**Assay Range:**

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-0.05 µmol/min/ml AP activity.

**Linearity of the Assay**

The following graph exhibits the linearity of the assay using wheat germ acid phosphatase.

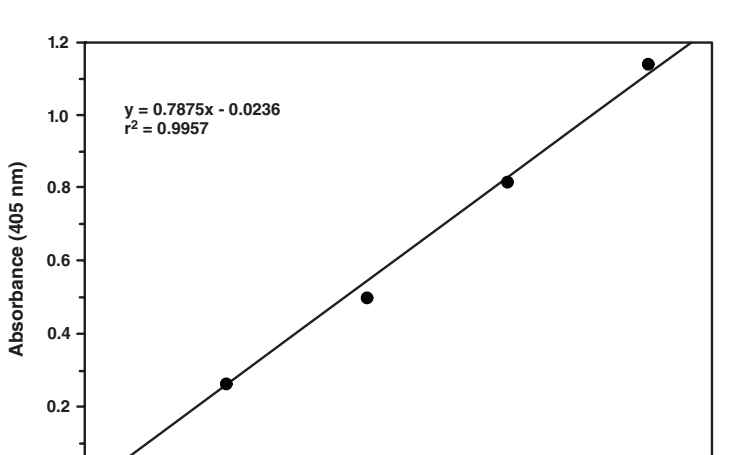



**Figure 3. Various dilutions of wheat germ acid phosphatase**

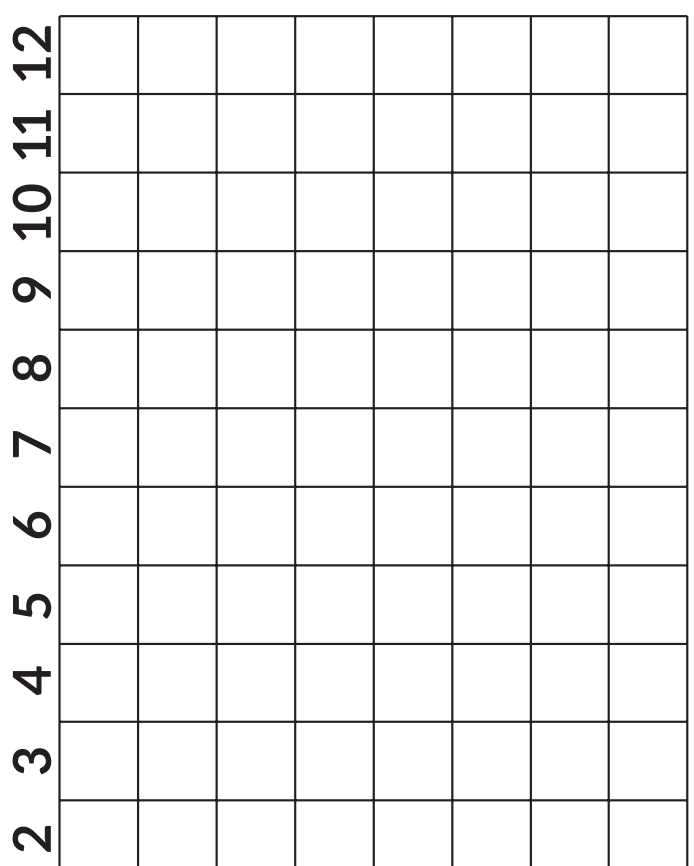
# RESOURCES

## Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|---|
| Erratic values; dispersion of duplicates/triplicates | A. Poor pipetting/technique<br>B. Bubble in the well(s) | A. Be careful not to splash the contents of the wells<br>B. Carefully tap the side of the plate with your finger to remove bubbles |
| Poor absorbance of both samples and controls | Plate was not incubated at 37°C | Re-assay the sample at 37°C |
| Acid Phosphatase was not detected in the sample | Sample was too dilute | Re-assay the sample using less of a dilution |
| Absorbance of sample fell above acceptable range (>1.5) | The sample is too concentrated | Dilute your sample with assay buffer and re-assay |

## References


1. Bull, H., Murray, P.G., Thomas, D., *et al.* Acid phosphatases. *J. Clin. Pathol: Mol. Pathol.* **55**, 65-72 (2002).
2. Yam, L.T. Clinical significance of the human acid phosphatases. *The American Journal of Medicine* **56**, 604-616 (1974).
3. Hudson, P.B., Brendler, H., and Scott, W.W. A simple method for the determination of serum acid phosphatase. *J. Urol.* **58**, 89-92 (1947).
4. Li, C.-Y., Chuda, R.A., Lam, W.K.W., *et al.* Acid phosphatases in human plasma. *J. Lab. Clin. Med.* **82(3)**, 446-460 (1973).
5. Gümüs, B., Lekili, M., Uyanik, B.S., *et al.* Serum levels of total acid phosphatase, prostatic acid phosphatase, total and free prostate-specific antigen in patients within chronic hemodialysis program. *Brazilian Journal of Urology* **27(2)**, 133-135 (2001).
6. Vaulbourdolle, M., Clavel, J.-P., Cynober, L., *et al.* Acid phosphatase and zinc in semen of subjects with no clinical evidence of prostatic disease. *Clin. Chem.* **31(6)**, 878-880 (1985).






## NOTES

## Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.