

Product Name: **Acid Phosphatase Activity Colorimetric Assay**

**Cat. #:** T2075-100 **Size:** 100 assays **Ship at** 4°C, **Store at** -20°C, **Avoid light** **Shelf Life:** 12 months

### Description

Acid phosphatases (AP) dephosphorylate phosphate groups from phosphate esters under acid conditions. Different acid phosphatase isozymes are found in different organs, and their serum levels are used as a diagnostic index for disease in the corresponding organs. Elevated prostatic acid phosphatase levels may indicate the presence of prostate cancer and elevated tartrate-resistant acid phosphatase levels may indicate bone disease. This Kit provides a high-sensitive, simple, and direct assay approach to measure AP activity in serum and other samples. It is suitable for research and drug discovery. The kit uses p-nitrophenyl phosphate (pNPP) as a phosphatase substrate which turns yellow ( $\lambda_{\max} = 405 \text{ nm}$ ) when dephosphorylated by AP. The kit can detect as low as 20  $\mu\text{U}$  acid phosphatase activity in samples.

**Applications** **Direct Assays:** Acid phosphatase in serum, plasma, urine, and other bio-samples.

### Key Features

Flexible: Suitable for colorimetric assay.

Accurate: Use 50  $\mu\text{L}$  samples. Detection ranges from 0.4-200  $\mu\text{U}$  in a 96-well plate for colorimetric assay.

Simple and high-throughput: Just load-incubate-Read. The kit can be used for a robust method.

Fast: less than 30 minutes.

### Kit Component

Assay Buffer: 10mL Substrate: 0.5mL Enzyme Standard Stock (2U/mL): 100  $\mu\text{L}$  Stop Solution: 10 mL

### Precaution

**Inhibitors of AP, such as tartrate, fluoride, EDTA, oxalate, and citrate, should be avoided in sample preparation.**

### Sample Preparations

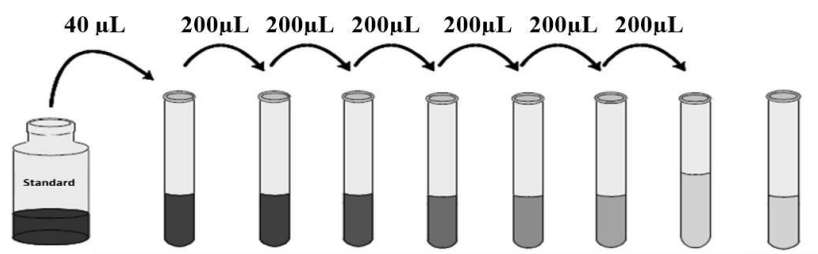
Sample Preparations: Serum, plasma, urine, semen, and cell culture media can be assayed directly.

Cells ( $1 \times 10^5$ ) or tissue ( $\sim 10 \text{ mg}$ ) can be homogenized in 150  $\mu\text{l}$  Assay Buffer, centrifuge to remove insoluble material at 13,000g, 3 minutes. The supernatant can be used as test sample for the assay testing.

### Standard Curve Preparations (Fig. 1)

1. Label 1.5mL tube from Std1 to 8. As below the diagram.
2. Add 360  $\mu\text{L}$  of 1x Assay Buffer to Std1, and 200  $\mu\text{L}$  to Std2 to 8.
3. Take 40  $\mu\text{L}$  of 1U/mL AP Standard Stock solution to Std1, then make 2x series dilution from Std2 through Std7 by transferring 200 $\mu\text{L}$  to the next concentration, Std8 is 1x Assay Buffer alone as a standard 0. The standard concentration range is 200, 100, 50, 25, 12.5, 6.25, 3.125  $\mu\text{M}$ , and 0.

**Fig. 1 Diagram for AP Standard Preparation**



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
<b>Assay Buffer (µL)</b>	360	200	200	200	200	200	200	200
<b>STD Addition</b>	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
<b>Addition (µL)</b>	40	200	200	200	200	200	200	
<b>Final Conc (mU/ml)</b>	200	100	50	25	12.5	6.25	3.125	0

### Assay Procedures

1. Add 45 µL of standard or sample to each well of a microplate in duplicate manner.
2. Add 5 µL substrate to each well, and incubate at room temperature for 15-60 minutes, protect from light.
3. Add 50 µL Stop Solution to terminate the reaction, and fully mix.
4. Measure OD value at test wavelength of 405 nm, and a reference wavelength of 630 nm in a plate reader.

### Related Products

ATP Colorimetric/Fluorometric Assay (T2010)      Glucose Oxidase Colorimetric/Fluorometric Assay (T2088)  
 ADP Colorimetric/Fluorometric Assay Kit (T2020)      β-Hexosaminidase Activity Assay (T2105)  
 Cytochrome C Oxidase Activity Assay (T2115)

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