Acid Phosphatase Activity Colorimetric Assay Kit

(Catalog# K411-500; 500 reactions; Store kit at –20° C)

I. Introduction:

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 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 the bone disease. BioVision's Acid Phosphatase Assay Kit is a high sensitivity, simple, direct and HTS-ready colorimetric assay designed to measure AP activity in serum and other samples. It is suitable for research and drug discovery. The kit uses *p*-nitrophenyl phosphate (*p*NPP) as a phosphatase substrate which turns yellow (λ_{max} = 405 nm) when dephosphorylated by AP. The kit can detect as low as 20 µU acid phosphatase activity in samples.

II. Kit Contents:

Components	K411-500	Cap Code	Part No.
AP Assay Buffer	100 ml	NM	K411-500-1
*p*NPP Substrate (10 TAB)	1 vial	Red	K411-500-2
AP Enzyme	1 vial	Green	K411-500-3
Stop Solution	10 ml	WM	K411-500-4

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

***p*NPP Solution:** Dissolve 2 tablets *p*NPP into 5.4 ml Assay Buffer to generate 5 mM *p*NPP. Two tablets are sufficient for 100 assays. **NEVER TOUCH THE TABLETS WITH BARE HANDS.** The *p*NPP solution is stable for 12 hours on ice.

AP Enzyme Solution: Reconstitute AP Enzyme with 1 ml Assay Buffer. **NEVER FREEZE!** The enzymes are stable for up to 2 months at 4°C after reconstitution.

Ensure that the Assay Buffer is at room temperature before use. Keep samples, *p*NNP substrate solution, and AP Enzyme on ice during the assay.

V. Acid Phosphatase Assay Protocol:

1. **Sample Preparations:**

   Inhibitors of AP, such as tartrate, fluoride, EDTA, oxalate, and citrate, should be avoided in sample preparation. Serum, plasma, urine, semen, and cell culture media can be assayed directly. Cells (1×10^5) or tissue (~10 mg) can be homogenized in 100 µl Assay Buffer, centrifuge to remove insoluble material at 13,000g, 3 minutes. Add test samples directly into 96-well plate, bring total volume to 80 µl with Assay Buffer.

   If samples contain color, it may interfere with O.D. 405 nm readings, use a sample background control. Add the same amount of sample into separate wells, bring volume to 80 µl. Add 20 µl stop solution and mix well to terminate AP activity in the sample.

2. Add 50 µl of *p*NPP Substrate Solution to each well containing the test samples and background controls. Mix well. Incubate the reaction for 60 min at 25°C, protect from light.

3. **Standard Curve:**

   Dilute 40 µl of the 5 mM *p*NPP solution with 160 µl Assay Buffer to generate 1 mM *p*NPP standard. Add 0, 4, 8, 12, 16, 20 µl into 96-well plate in duplicate to generate 0, 4, 8, 12, 16, 20 nmol/well *p*NPP standard. Bring the final volume to 120 µl with Assay Buffer.

   Add 10 µl of AP enzyme solution to each well containing the *p*NPP standard. Mix well. The AP enzyme will convert *p*NPP substrate to an equal amount of colored *p*-Nitrophenol (*p*NP). Incubate the reaction for 60 min at 25°C, protect from light.

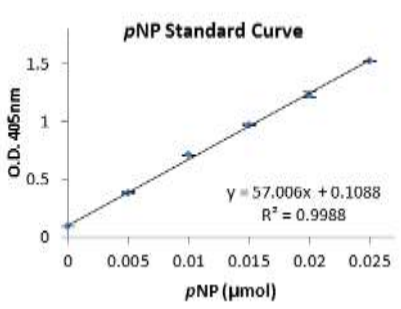
4. Stop all reactions by adding 20 µl Stop Solution into each standard and sample reaction except the sample background control reaction (since 20 µl Stop Solution has been added into the background control when the sample was prepared in step 1), gently shake the plate. Measure O.D. 405 nm in a micro-plate reader.

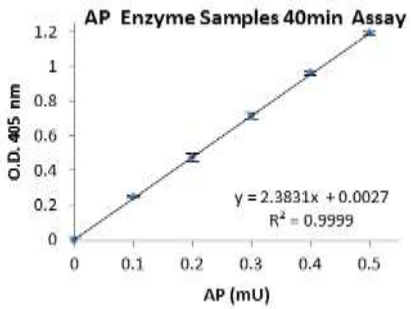
5. **Calculation:** Correct background by subtracting the value derived from the 0 standard from all the standards, samples and sample background controls (The background reading can be significant and must be subtracted from sample readings). Plot *p*NP standard Curve. Apply sample readings to the standard curve to get the amount of *p*NP generated by AP sample. AP activity of the test samples can then be calculated:

   AP activity (U/ml) = A/V/T

   Where A is amount of *p*NP generated by samples (in µmol).
   V is volume of sample added in the assay well (in ml).
   T is reaction time (in minutes)

   Unit Definition: One unit of AP is the amount of enzyme causing the hydrolysis of one micromole of *p*NPP to *p*NP per minute at pH 5.0 and 25°C.





RELATED PRODUCTS:

Alkaline Phosphatase Assay Kit
Phosphate Fluorescence Assay Kit
NAD/NADH Quantification Kit
Lactate/Pyruvate Assay Kit/ II
Ammonia Assay Kit
Glucose Assay Kit
ADP/ATP Ratio Assay Kit
Phosphate Colorimetric Assay Kit
Uric Acid Assay K
Uric Acid Assay Kit
Glutamate Assay Kit
Fatty Acid Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		