

Acid Phosphatase Activity Colorimetric Assay Kit

(Catalog# BN00664; 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. Assay Genie'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 (pNPP) as a phosphatase substrate which turns yellow ($\lambda_{\text{max}} = 405\text{ nm}$) when dephosphorylated by AP. The kit can detect as low as 20 μU acid phosphatase activity in samples.

II. Kit Contents:

Components	BN00664	Cap Code	Part No.
AP Assay Buffer	100 ml	NM	BN00664-1
pNPP Substrate (10 TAB)	1 vial	Red	BN00664-2
AP Enzyme	1 vial	Green	BN00664-3
Stop Solution	10 ml	WM	BN00664-4

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:

pNPP Solution: Dissolve 2 tablets pNPP into 5.4 ml Assay Buffer to generate 5 mM pNPP. Two tablets are sufficient for 100 assays. **NEVER TOUCH THE TABLETS WITH BARE HANDS.** The pNPP 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, pNPP 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 ($\sim 10\text{ mg}$) 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 pNPP 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 pNPP solution with 160 μl Assay Buffer to generate 1 mM pNPP 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 pNPP standard. Bring the final volume to 120 μl with Assay Buffer.

Add 10 μl of AP enzyme solution to each well containing the pNPP standard. Mix well. The AP enzyme will convert pNPP substrate to an equal amount of colored *p*-Nitrophenol (pNP). 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 pNP standard Curve. Apply sample readings to the standard curve to get the amount of pNP generated by AP sample. AP activity of the test samples can then be calculated:

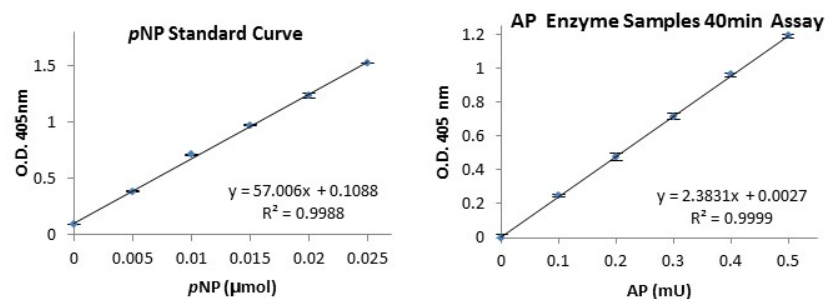
$$\text{AP activity (U/ml)} = A/V/T$$

Where A is amount of pNP 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 pNPP to pNP per minute at pH 5.0 and 25°C .



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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		