

## Product Information

### Alkaline Phosphatase Activity Colorimetric Assay Kit

#### I. Kit Contents:

Components	K2075-500 500 assays	Cap Color	Part Number
ALP Assay Buffer	100 ml	NM	K2075-C-1
pNPP (10 TAB)	1 vial	Red	K2075-C-2
ALP Enzyme	1 vial	Green	K2075-C-3
Stop Solution	10 ml	WM	K2075-C-4

#### II. Introduction:

Alkaline phosphatase (ALP) is a hydrolase enzyme that removes phosphate groups from nucleotides proteins and alkaloids. Changes in ALP level and activity are associated with various disease states in the bone and liver.

The Alkaline Phosphatase Activity Colorimetric Assay Kit provides a highly sensitive and convenient way for detection of ALP activity based on colorimetric method in serum and biological samples. The kit contains 10 substrate tablets providing convenience for multiple usages. The assay utilizes p-nitrophenyl phosphate (pNPP) as a phosphatase substrate which produces yellow ( $\lambda_{\text{max}} = 405 \text{ nm}$ ) when dephosphorylated by ALP that can be easily detected using a spectrophotometer or an ELISA plate reader. The assay can detect 10-250  $\mu\text{U}$  ALP in various samples.

#### III. Storage and Handling:

Store the kit at  $-20^\circ\text{C}$ , 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 make 5 mM work solution. Two tablets are sufficient for 100 assays. Never Touch Tablets With Bare Hands. The pNPP solution is stable for 12 hours on ice.

**ALP Enzyme:** Reconstitute ALP Enzyme with 1 ml Assay Buffer. DO Not Freeze! The enzymes are stable for up to 2 month at  $4^\circ\text{C}$  after reconstitution.

**Note:** Ensure that the Assay Buffer is at room temperature before use. Keep samples, ALP Enzyme and pNPP solution on ice during the assay.

#### V. Alkaline Phosphatase Assay Protocol:

##### 1. Sample Preparations:

Inhibitors of ALP, such as EDTA, oxalate, fluoride, and citrate should be avoided in sample preparation. Serum and plasma should be diluted 10 times; cell culture media can be measured directly. To measure intracellular ALP, washed cells ( $1 \times 10^5$ ) can be homogenized in the Assay Buffer, centrifuge to remove insoluble material at 13,000g for 3 minutes. Add different volume of samples into 96-well plate; bring the total volume to 80  $\mu\text{l}$  with Assay Buffer.

Colored samples may interfere with  $\text{OD}_{405 \text{ nm}}$  readings, so use a sample background control. Add the same amount of sample into separate wells, bring volume to 80  $\mu\text{l}$ . Add 20  $\mu\text{l}$  stop solution and mix well to terminate ALP activity in the sample.

2. Add 50  $\mu$ l of the 5 mM pNPP 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  $\mu$ l of the 5 mM pNPP solution with 160  $\mu$ l Assay Buffer to generate 1 mM pNPP standard. Add 0, 4, 8, 12, 16, 20  $\mu$ l into 96-well plate in duplicate to generate 0, 4, 8, 12, 16, 20 nmol/well pNPP standard. Bring the final volume to 120  $\mu$ l with Assay Buffer. Add 10  $\mu$ l of ALP enzyme solution to each well containing the pNPP standard. Mix well. The ALP 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  $\mu$ l Stop Solution into each standard and sample reaction except the sample background control reaction (since 20  $\mu$ l Stop Solution has been added to the background control when prepared in step 1), gently shake the plate. Measure O.D. at 405 nm in a micro plate reader.

5. Calculation: Correct background by subtracting the value derived from the 0 standards from all standards, samples and sample background control (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 ALP sample. ALP activity of the test samples can then be calculated:

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

Where A is amount of pNP generated by samples (in  $\mu$ mol).

V is volume of sample added in the assay well (in ml).

T is reaction time (in minutes).

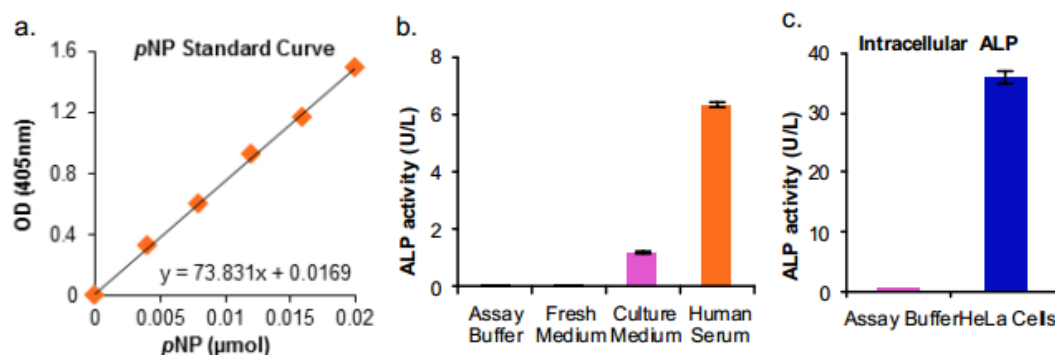


Figure: a. pNP Standard Curve. b. Measurement of ALP activity in fresh medium (80  $\mu$ l, without culturing), 3-day old HeLa cell culture medium (80  $\mu$ l), and human serum (80  $\mu$ l, 1:10 diluted). c. Measurement of ALP activity in HeLa cells:  $5 \times 10^4$  HeLa cells were homogenized in 1 ml of Assay Buffer, diluted 1:10 in Assay Buffer and 80  $\mu$ l was used to measure ALP activity. Assays were performed following the kit protocol.

### General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Use of a different buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates</li> </ul>
Samples with	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> </ul>

erratic readings	<ul style="list-style-type: none"> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed, deproteinize samples</li> <li>• Use fresh samples or store at correct temperatures till use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer data sheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

**For research use only! Not to be used in humans.**

## Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit <http://www.apexbt.com/> or contact our technical team.

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