ab83367 – Acid Phosphatase Assay Kit (Colorimetric)

For the screening of Acid Phosphatase activity.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab83367

Storage and Stability
On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
AP Assay Buffer	100 mL	-20°C
pNPP Substrate/pNPP (10 tablets)	1 vial	-20°C
AP Enzyme	1 vial	-20°C
Stop Solution I/Stop Solution	10 mL	-20°C

Materials Required, Not Supplied
These materials are not included in the kit, but will be required to successfully utilize this assay:
- Temperature-controlled plate reader
- 96-well clear plate with flat bottom, UV transparent plate is preferred.

Reagent Preparation
- Before using the kit, spin the tubes prior to opening.
- Ensure that the Assay Buffer is at room temperature before use.
- Keep samples, pNNP substrate solution, and AP Enzyme on ice during the assay

pNPP Solution: Dissolve 2 tablets pNPP Substrate/pNPP into 5.4 ml Assay Buffer to generate 5 mM pNPP. Two tablets are sufficient for 100 assays. The pNPP Substrate/pNPP solution is stable for 12 hours on ice.
Δ Note: DO NOT TOUCH THE TABLETS WITH BARE HANDS.

AP Enzyme Solution: Reconstitute AP Enzyme with 1 ml Assay Buffer. DO NOT FREEZE! The enzymes are stable for up to 2 months at 4°C after reconstitution.

Assay Protocol
Sample Preparations:

1. 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 I/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.

Standard Curve

3. Dilute 40 µl of the 5 mM pNPP Substrate/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 I/Stop Solution into each standard and sample reaction except the sample background control reaction (since 20 µl Stop Solution I/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

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:

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

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.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)