ab83369 Alkaline Phosphatase Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Alkaline Phosphatase (ALP) activity in various samples. For research use only – not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab83369
(use abcam.cn/ab83369 for China, or abcam.co.jp/ab83369 for Japan)

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.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
ALP Assay Buffer I/ALP Assay Buffer	100 mL	-20°C	-20°C
pNPP Substrate/pNPP	10 tablets	-20°C	4°C
Enzyme Mix II/ALP Enzyme	1 vial	-20°C	4°C
Stop Solution I/Stop Solution	10 mL	-20°C	-20°C

Storage and Stability:
Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Refer to list of materials supplied for storage conditions of individual components before and after prep. Aliquot components in working volumes before storing at the recommended temperature.

Materials Required, Not Supplied:
- Microplate reader capable of measuring absorbance at OD 405 nm
- Double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)

Reagent Preparation:
Briefly centrifuge small vials at low speed prior to opening.

1. **ALP Assay Buffer I/ALP Assay Buffer:**
   Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.
2. **ALP Enzyme:**
   Reconstitute ALP Enzyme with 1mL ALP Assay Buffer I/Assay Buffer. Keep on ice during the assay. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store aliquots at 4°C (do not freeze enzyme once reconstituted). Use within 2 months.
3. ***pNPP* Solution:**
   Δ **Note:** never touch the tablets with bare hands.
   Reconstitute 2 *p*NPP tablets in 5.4 mL ALP Assay Buffer I/Assay buffer to make a 5 mM solution; this is enough for 100 assays. The *p*NPP solution is stable for 12 hours on ice. Store unused tablets at -20°C.
4. **Stop Solution:**
   Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Standard Preparation:
- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare a 1mM pNPP standard by diluting 40µL pNPP Substrate/pNPP 5mM Standard in 160 µL of ALP Assay Buffer I/Assay Buffer.
2. Using 1mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard#	*p*NPP 1 mM Standard (µL)	ALP Assay Buffer I/Assay Buffer (µL)	Final volume standard in well (µL)	End amount *p*NPP in well (nmol/well)
1	0	300	120	0
2	10	290	120	4
3	20	280	120	8
4	30	270	120	12
5	40	260	120	16
6	50	250	120	20

Each dilution has enough amount of standard to set up duplicate readings (2 x 120 µL).

Sample Preparation:
- We recommend performing several dilutions of sample to ensure readings are within the standard range.
- We recommend using fresh samples. If you cannot perform the assay at the same time, snap freeze samples in liquid nitrogen and store at -80°C.

1. Cell lysates:
1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^5 cells).
1.2 Wash cells with cold PBS.
1.3 Resuspend cells in 50 µL of ALP Assay Buffer I/Assay Buffer.
1.4 Homogenize the cells using a Dounce homogenizer (10 – 50 passes) on ice.
1.5 Centrifuge samples at 4°C at top speed for 15 minutes in a cold microcentrifuge to remove any insoluble material.
1.6 Collect supernatant and transfer to a new tube.
1.7 Keep sample on ice.

2. Tissue lysates:

2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg tissue).
2.2 Homogenize in 4 – 6 volumes of ALP Assay Buffer I/Assay Buffer using a Dounce homogenizer (10 – 50 passes) on ice.
2.3 Centrifuge samples at 4°C at top speed for 15 minutes in a cold microcentrifuge to remove any insoluble material.
2.4 Collect supernatant and transfer to a new tube.
2.5 Keep sample on ice.

3. Plasma, Serum and Urine (and other biological fluids):
Plasma and serum samples can be tested directly; they do not require additional sample preparation. Dilute sample 10 times in ALP Assay Buffer I/Assay Buffer. However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

4. Cell culture medium:
Cell culture medium can be analyzed directly; it does not require additional sample preparation. However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample. For cell culture medium phenol red-free medium is recommended.
Δ **Note**: We suggest using different volumes of sample to ensure readings are within the standard curve range.

Assay Procedure:

– Equilibrate all materials and prepared reagents to room temperature prior to use.
– We recommend that you assay all standards, controls and samples in duplicate.
– Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ **Note:** Set up Sample Background Controls for colored samples, as the color may interfere with the reading.

1. **Reaction wells set up:**
   Standard wells = 120 µL standard dilutions.
   Sample wells = 2-80 µL samples (adjust volume to 80 µL/well with ALP Assay Buffer I/Assay Buffer).
   Sample Background Control wells (for colored samples only) = 2- 80 µL samples (adjust volume to 80 µL/well with ALP Assay Buffer I/Assay Buffer).
2. **ALP Reaction:**
   2.1 Add 20 µL Stop Solution to Sample Background Control wells to terminate ALP activity in these samples. Mix well by pipetting up and down.
   2.2 Add 50 µL of 5 mM *p*NPP Solution (Reagent Prep step 3) to each well containing Sample and Background Sample Controls. Do not add solution to Standard wells.
   2.3 Add 10 µL of ALP enzyme solution to each pNPP Standard well. Mix by pipetting up and down. Add
   2.4 Incubate plate at 25°C for 60 minutes protected from light. The enxyme will convert *p*NPP substrate to an equal amount of colored p-Nitrophenol (*p*NP).
   2.5 Stop reaction in Sample wells and Standard wells by adding 20 µL Stop Solution.

Δ **Note:** Do not add Stop Solution to Sample Background Control wells as the Stop Solution has already been added to the control when prepared in Step 2.1.

3. **Measurement:**
   3.1 Gently shake the plate.
   3.2 Measure output at OD 405 nm on a microplate reader.

Calculations:

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. **Standard curve calculation:**
   1.1 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
   1.2 Average the duplicate reading for each standard.
   1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).
2. **Measurement of ALP activity in the sample**:
   2.1 Average the duplicate reading for each sample.
   2.2 If significant and applicable, determine the background corrected change in absorbance intensity for each well of sample by subtracting the OD value of the background control.
   2.3 ALP activity (µmol/min/mL or U/mL) in the test samples is calculated as:

$$ALP\ Activity = \left(\frac{B}{T * V}\right) * D$$

   Where:
   B = amount of *p*NP in sample well calculated from standard curve (µmol).
   T = reaction time (minutes).
   V = original sample volume added into the reaction well (mL).
   D = sample dilution factor.

Unit Definition:
All the Units mentioned in this protocol are Glycine Units.
Glycine Units: The amount of enzyme causing the hydrolysis of one micromole of pNPP Substrate/pNPP per minute at pH 9.6 and 25°C (glycine buffer).
DEA Units: The amount of enzyme causing the hydrolysis of one micromole of pNPP Substrate/pNPP per minute at pH 9.8 and 37°C (diethanolamine buffer).
Unit Conversion: One Glycine unit as described above is equivalent to approximately three DEA units. This reaction system is in Glycine buffer.

Interferences: These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

– Inhibitors of ALP such as EDTA, oxalate, fluoride and citrate should be avoided in sample preparation.

FAQs:

Q. Can this kit be used with isolated protein samples that contain protease inhibitor? Are there any other components that could affect the performance of this kit when one uses protease inhibitors?
A. The only chemicals you need to be wary of are EDTA, oxalate, fluoride, and citrate.
Q. Once the pNPP solution has been prepared, can I freeze the pNPP solution (e.g. at -20°C) so that I can use it later? If so, what is the maximum time I can store it at -20°C?
A. The pNPP solution should be used within 12 hours of making. Once you collect all samples, you can make the fresh pNPP solution and use it right away.
Q. Can samples be measured at different time points? During the sample preparation, once I have added ALP ALP Assay Buffer I/assay buffer to my cell samples, can I then freeze these samples to -20°C?

A. Samples homogenized in the ALP Assay Buffer I/assay buffer can be frozen in aliquots at - 80°C until analysis.

Q. Can the incubation steps for pNPP Substrate/pNPP and the ALP enzyme be combined into a single one hour incubation step? Or is it necessary to let the two incubations take place separately.

A. Yes, you can do the incubation simultaneously.

Q. I was thinking to keep the volume (and number) of cells stable and change the dose concentration of the drug I am testing with this assay. Why do you recommend different cell volumes?

A. We are not taking different volume of cells, but different volume of the cell homogenate. Plate equal number of cells in each well, treat them with the drugs, then trypsinize out the cells, get the cell pellet, wash with ice cold PBS, homogenize the resultant pellet in the ALP Assay Buffer I/assay buffer, take the supernatant from that and use different volumes of this supernatant for the subsequent assay.

Q. How do we normalize our final readings?

A. If you are beginning with variable number of cells, you can normalize against the total cell number or protein quantity used.

Q. We are looking for a kit to detect secreted alkaline phosphatase (SEAL) reporter gene in serum samples. Can we use this kit?

A. If you want to assay for the gene, you need to do DNA isolation from the serum and use a PCR based reaction to detect the specific gene. This assay is to detect the enzyme's activity. A DNA isolation kit from serum samples (for example, DNA Isolation Kit – Plasma/Serum (ab156893)) and some SEAL specific primers would be ideal for you.

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