Acid Phosphatase Colorimetric Assay Kit

Product description

Acid phosphatase (ACP) is an acid hydrolase abundant in lysosomes and widely used as a lysosome-specific biomarker. It is divided into tartrate-sensitive (lysosome - originated) and fluoride-sensitive isoforms (found in erythrocytes and macrophages). ACP activity is low in normal serum, plasma and urine, but high in semen. Altered ACP activity occurs under pathological conditions such as prostate cancer bone metastasis, Gaucher disease and hemolytic disorders, making it a valuable biochemical marker for lysosomal function, cellular injury and tumor progression in cell biology and reproductive-related research.

This colorimetric kit uses p-nitrophenyl phosphate (pNPP) as substrate. Under acidic conditions, ACP hydrolyzes pNPP into p-nitrophenol (pNP). After alkalization, yellow-colored pNP is quantified by absorbance at 405 nm, with signal intensity proportional to ACP activity. The assay requires minimal sample pretreatment and is compatible with cells, tissues, plasma, serum and urine samples.

Components and storage conditions

Components \ Size	120 Assays	Storage
ACP Assay Buffer	15 mL	-20°C
pNPP Substrate	2 Vials	-20°C away from light
pNP (10 mM)	0.1 mL	-20°C away from light
Stop Solution	20 mL	-20°C
Shipping: Blue ice	**Shelf life:** 1 year	

Experimental operation

1. Preparation before the experiment

   1) Allow the ACP Assay Buffer and Stop Solution to equilibrate to room temperature.

   2) Dissolve a vial of pNPP Substrate in 2.694 mL ACP Assay Buffer to obtain pNPP solution. One vial of pNPP solution is sufficient for 50 assays. The reconstituted pNPP solution should be kept on ice and used within 6 h, or temporarily stored at -20°C. To avoid kit waste, prepare sufficient samples to run together.

   3) Standard preparation: Dilute 10 µL pNP (10 mM) in 190 µL ACP Assay Buffer to obtain pNP (0.5 mM).

Note: Upon first use, except for ACP Assay Buffer, aliquot all other components to avoid repeated freeze-thaw cycles.

2. Sample preparation

1) Cell samples: Collect 1 × 10^6 cells and add 100 µL of suitable lysis buffer. Cell Lysis Buffer for WB and IP without inhibitors (Cat. No. K1124) is recommended. Homogenize if necessary. Centrifuge and retain the supernatant.

2) Tissue sample: Weigh 10 mg of tissue, add 100 µL of suitable lysis buffer, and homogenize on ice. Cell Lysis Buffer for WB and IP without inhibitors (Cat. No. K1124) is recommended. Centrifuge and retain the supernatant.

3) Serum/plasma/urine: These samples can generally be used directly. For serum or plasma, to eliminate interference from the sample, it is recommended to set up a control group containing sample but no substrate.

Note:

a) When lysing cells or tissues, lysis buffer must be free of phosphatase inhibitors. During sample preparation, avoid acid phosphatase inhibitors including tartrates, fluorides, EDTA, oxalates and citrates.

b) Try to use fresh samples for testing. If immediate testing is not possible, store prepared samples at -80°C and avoid repeated freeze-thaw cycles.

3. Sample dilution (optional): If the sample contains highly active acid phosphatase, dilute the sample with the lysis buffer, PBS or ACP Assay Buffer. If ACP Assay Buffer is used for dilution, it is important to reserve sufficient ACP Assay Buffer for experimental testing.

4. ACP enzyme activity testing

1) Refer to the table below to set up groups in 96-well plates. Add 0, 4, 8, 16, 24, 32, and 40 µL pNP standard into a 96-well plate. Bring the final volume to 80 µL with ACP Assay Buffer. Generally, 40 µL of sample can be loaded per well. After adding all components, mix gently by repeated pipetting, or using a plate shaker.

	Blank	**Sample**	**Standards**
ACP Assay Buffer	40 µL	(40-X) µL	(80-Y) µL
pNPP	40 µL	40 µL	-
pNP (0.5 mM)	-	-	Y µL
Sample	-	X µL	-

Note: After adding pNPP to the sample, due to the overall acidic environment of the solution, the reaction mixture may turn cloudy. This does not interfere with measurements; the solution will turn clear once Stop Solution is added.

2) Incubate at 37°C in the dark for 5-10 min. The optimal incubation time varies with experiments and may be extended up to 30 min.

3) Add 160 μL of Stop Solution to each well and mix well to terminate ACP enzyme activity. At this point, different shades of yellow appear in the wells of the standard and samples with ACP enzyme activity.

4) Place the plate in a microplate reader and shake for several seconds, then measure absorbance at 405 nm (readings can also be acquired within 400-415 nm). Readings remain valid for several hours if measurement cannot be carried out immediately.

5. ACP enzyme activity calculation

1) Definition of ACP activity: One unit of ACP activity is defined as the amount of enzyme that hydrolyzes pNPP to generate 1 μmol pNP per minute at pH 4.8 and 37°C.

2) Subtract blank readings from all raw absorbance readings to obtain corrected values. A standard curve is established based on these corrected values.

3) Apply sample readings to the standard curve to obtain the amount of pNP in the reaction system.

4) Refer to the following formula to calculate ACP enzyme activity

$$ACP\ activity\ (mU/mL) = \frac{B}{\Delta T \times V} \times D$$

Where:

B is the amount of pNP in the reaction system calculated from the standard curve (nmol)

ΔT is the reaction time (min)

V is the volume of sample added to the reaction system (mL)

D is the dilution factor of the sample before being added to the reaction system

Notes

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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