

Alkaline Phosphatase (ALP) Activity Assay Kit (pNPP method)

A1491772

Storage: -20°C. Store in the dark.

Introduction:

This kit enables rapid and convenient detection of endogenous alkaline phosphatase (ALP) activity in cell or tissue lysates, serum, plasma, urine, and other sample types. Alkaline phosphatase, also known as alkaline phosphomonoesterase, catalyzes the hydrolysis of phosphate esters under alkaline conditions. Major ALP isozymes include intestinal, tissue-nonspecific, and placental alkaline phosphatases. With the exception of placental ALP, most isozymes are heat-labile.

Para-Nitrophenyl phosphate (pNPP) is a widely used chromogenic substrate for phosphatases. Under alkaline conditions, ALP hydrolyzes pNPP to generate *para*-nitrophenol (p-nitrophenol), which yields a yellow product in basic solution with maximum absorbance at 405 nm. The intensity of the yellow color is proportional to ALP activity, allowing quantitative measurement via spectrophotometry.

This kit provides sufficient reagents for 100 assays.

Kit Contents

A1491772	Component	100T	Storage
A1491772A	Assay Buffer	50 mL	-20°C
A1491772B	Chromogenic Substrate	2 tubes	-20°C. Store in the dark.
A1491772C	p-nitrophenol	1 mg	-20°C. Store in the dark.
A1491772D	Stop Solution	12 mL	-20°C.

Instructions for Use:

1. Reagent Preparation: Bring all reagents to room temperature before use.
 - 1.1. Chromogenic Substrate Solution: Dissolve one tube of substrate in 2.51 mL Assay Buffer. Mix thoroughly and keep on ice. Use within 6 hours.
 - 1.2. 10 mM p-Nitrophenol Stock Solution: Dissolve 1 mg p-nitrophenol in 719 μ L ultrapure water to obtain 10 mM solution. Store at -20°C.
 - 1.3. Standard Working Solution: Dilute 10 μ L of 10 mM p-nitrophenol solution with Assay Buffer to 0.2 mL (final concentration: 0.5 mM).
2. Sample Preparation:
 - 2.1. Cell or Tissue Lysates: Lyse cells or tissues using an appropriate lysis buffer (without

phosphatase inhibitors). Centrifuge and collect supernatant. Avoid repeated freeze-thaw cycles. Western & IP Lysis Buffer (without protease inhibitors) is recommended.

- 2.2. Plasma, Serum, Urine: These can be used directly. Include a no-substrate control for plasma/serum to account for background color. Do not use EDTA or citrate anticoagulants. Urine may typically be used directly. Avoid repeated freeze-thaw cycles.
- 2.3. Sample Dilution: If ALP activity is high, dilute samples with lysis buffer, PBS, or Assay Buffer. Ensure sufficient Assay Buffer remains for the assay.
3. Equilibrate substrate solution at 37°C and set microplate reader to 405 nm.
4. Set up blank, standard, and sample wells in a 96-well plate as below. Standard volumes: 4, 8, 16, 24, 32, 40 µL. Sample volume: typically 50 µL. Reduce volume or dilute if ALP activity is too high.

Reagent	Blank	Standard	Sample
Assay Buffer	50 µL	(100-X) µL	(50-Y) µL
Substrate Solution	50 µL	–	50 µL
Sample	–	–	Y µL
Standard Working Solution	–	X µL	–

5. Mix gently by pipetting or using a plate shaker.
6. Incubate at 37°C for 5–10 min (extend to 30 min for low-activity samples).
7. Add 100 µL Stop Solution per well to terminate the reaction. A yellow color will develop in positive wells.
8. Measure absorbance at 405 nm

Definition of ALP Activity Unit:

- One DEA unit is defined as the amount of enzyme required to produce 1 µM *p*- nitrophenol per minute at 37°C in pH 9.8 diethanolamine (DEA) buffer.
- One Glycine unit is defined as the amount of enzyme required to produce 1 µM *p*- nitrophenol per minute at 25°C in pH 9.6 glycine buffer.
- One Glycine unit ≈ 3 DEA units. This kit measures DEA units.

Calculation of ALP Activity:

1. Standard working solution concentration: 500 µM.
2. Standard volumes correspond to final amounts of 20, 40, 80, 120, 160, and 200 units (for 5-min incubation).
3. Keep incubation time consistent for all samples (e.g., 5 min).
4. Generate standard curve: (A_{405} Standard – A_{405} Blank) → regression equation.
5. Calculate sample value: (A_{405} Sample – A_{405} Blank).
6. Interpolate sample value into standard curve to determine ALP activity.

Precautions:

1. For absolute quantification, precisely time the reaction. Use longer incubation (e.g., 30 min) to reduce operational error. Dilute high-activity samples appropriately.
2. Avoid ALP inhibitors such as EDTA, fluoride, and citrate in samples.
3. Assay Buffer and *p*-nitrophenol are hazardous. Stop Solution is corrosive—handle with care.
4. It is recommended to test 1–2 samples initially as a pilot experiment.
5. Wear appropriate personal protective equipment (lab coat, gloves) while handling reagents.
6. For research use only.

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