TECHNICAL MANUAL

Alkaline Phosphatase (ALP) Activity Assay Kit (PNPP)

- **SKU CODE:** MAES0023
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubation
- Stop reaction
- Measure absorbance at 405 nm

2. Intended use

This kit can be used to measure alkaline phosphatase (ALP) activity in animal tissue, serum (plasma) and cell samples.

3. Detection principle

Under alkaline conditions, alkaline phosphatase catalyzes the hydrolysis of p-nitrobenzene phosphate disodium to produce p-nitrophenol and phosphoric acid. Under strong alkaline conditions, p-nitrophenol is bright yellow and has a maximum absorption peak at 405 nm. Therefore, the activity of ALP can be calculated by measuring the OD value at 405 nm.

4. Kit components & storage

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
Reagent 1	Buffer Solution	60 mL x 1 vial	60 mL x 5 vials	-20 °C, 12 months
Reagent 2	Substrate	Powder x 2 vials	Powder x 10 vials	-20 °C, 12 months, shading light
Reagent 3	10 mmol/L p-Nitrophenol Standard Solution	0.4 mL x 1 vial	2 mL x 1 vial	-20 °C, 12 months shading light
Reagent 4	Stop Solution	12 mL x 1 vial	60 mL x 1 vial	-20 °C, 12 months
	Microplate	96 wells	/	No requirement

Item	Component	Size 1 (96 T)	Size 2 (500 Assays)	Storage
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Micropipettor, Microplate reader (400-415 nm, optimum wavelength: 405 nm), Incubator, Water bath

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 3 mL of buffer solution. Store at -20 °C for 24 hours protected from light.
3. **Preparation of 500 μmol/L standard:** Dilute 35 μL of 10 mmol/L p-Nitrophenol standard solution with 665 μL of buffer solution. Mix well to dissolve. The 500 μmol/L standard should be prepared immediately before use.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 500 μmol/L standard solution with buffer solution to serial concentrations. The recommended dilution gradient is as follows: 500, 400, 320, 240, 160, 80, 40, 0 μmol/L. Concentration (μmol/L): 0, 40, 80, 160, 240, 320, 400, 500 500 μmol/L standard (μL): 0, 16, 32, 64, 96, 128, 160, 200 Buffer solution (μL): 200, 184, 168, 136, 104, 72, 40, 0

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for one month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold normal saline (0.9% NaCl).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C.

4. Centrifuge at 10,000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cell (adherent or suspension) sample:

1. Harvest the number of cells needed for each assay (initial recommendation 3 x 10^6 cells).

2. Wash cells with normal saline (0.9% NaCl).

3. Homogenize 3 x 10^6 cells in 200-400 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 10,000 xg for 10 min at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

Human serum: 10-20

Mouse serum: 20-30

Rat serum: 10-20

Mouse plasma: 10-20

HepG2 supernatant: 8-12

10% Rat kidney tissue homogenate: 400-1200

10% Mouse liver tissue homogenate: 10-15

10% Rat lung tissue homogenate: 50-100

10% Mouse brain tissue homogenate: 30-50

Human urine: 1

Note: The diluent is buffer solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

Substrate working solution and standard should be stored protected from light.

9. Operating steps

1. Standard well: Add 50 µL of standards with different concentrations into the standard wells. Sample well: Add 50 µL of sample into the sample wells. Control well: Add 50 µL of buffer solution into the control wells.
2. Add 50 µL of substrate working solution to the control wells and sample wells. Add 50 µL of buffer solution to the standard wells.
3. Incubate at 37 °C for 10 min.
4. Add 100 µL of stop solution to each well.
5. Mix thoroughly for 5 s with microplate reader. Measure the OD values of each well at 405 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) sample:

Definition: The amount of 1 µmol p-nitrophenol produced by 1 L serum (plasma) per minute when catalyzing the substrate at 37 °C is defined as 1 activity unit.

ALP activity (U/L) = (ΔA - b) ÷ a ÷ T × f

2. Tissue sample:

Definition: The amount of 1 µmol p-nitrophenol produced by 1 g tissue protein per minute when catalyzing the substrate at 37 °C is defined as 1 activity unit.

ALP activity (U/gprot) = (ΔA - b) ÷ a ÷ T × f ÷ C_{pr}

3. Cell sample:

Definition: The amount of 1 µmol p-nitrophenol produced by 1 g cell protein per minute when catalyzing the substrate at 37 °C is defined as 1 activity unit.

ALP activity (U/gprot) = (ΔA - b) ÷ a ÷ T × f ÷ C_{pr}

[Note]

ΔA: Absolute OD value, OD_{Sample} - $OD_{Control}$

f: Dilution factor of sample before test

T: Reaction time, 10 min

C_{pr}: Concentration of protein in sample, gprot/L

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.80	12.00	35.00
%CV	1.5	1.0	1.1

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

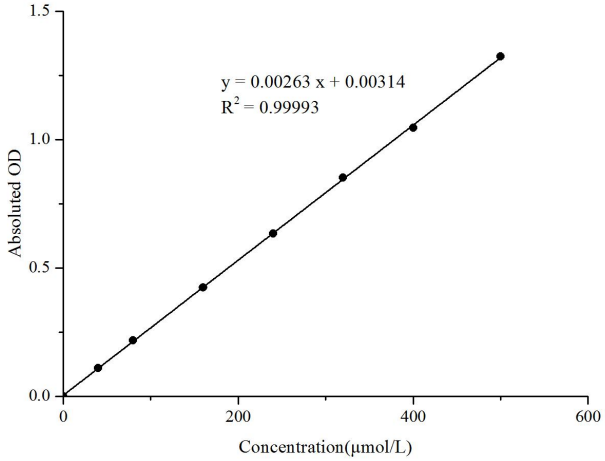
Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.80	12.00	35.00
%CV	4.3	4.8	4.7

Sensitivity

The analytical sensitivity of the assay is 0.27 U/L ALP. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μmol/L)	Average OD	Absolute OD
0	0.050	0.000
40	0.159	0.110
80	0.267	0.217
160	0.474	0.424
240	0.684	0.634
320	0.902	0.852
400	1.095	1.045
500	1.374	1.324

12. Appendix II Example Analysis

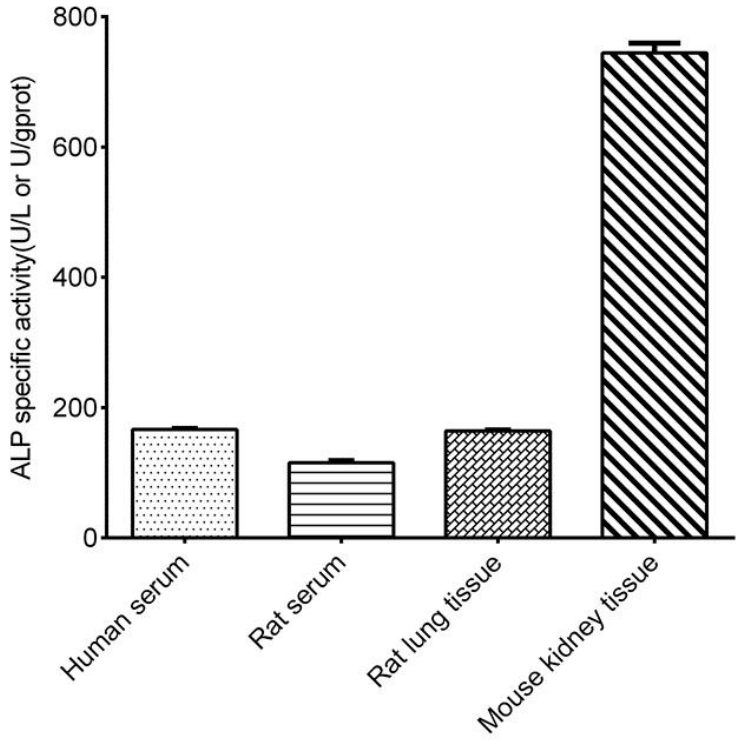
Example analysis:

For human serum, dilute human serum with buffer solution 10-fold, take 50 µL of diluted samples and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0027x - 0.0051, the average OD value of the control is 0.099, the average OD value of the sample is 0.544, and the calculation result is:

ALP activity (U/L) = (0.544 - 0.099 + 0.0051) ÷ 0.0027 ÷ 10 × 10 = 166.70 U/L

Detect human serum (diluted 10-fold), rat serum (diluted 10-fold), 10% rat lung tissue homogenate (the concentration of protein is 3.18 gprot/L diluted 80-fold) and 10% mouse kidney tissue homogenate (the concentration of protein is 6.33 gprot/L diluted 400-fold) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.