Alkaline Phosphatase Colorimetric Activity Assay Kit

Item No. 701710

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TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Safety Data
4 Precautions
4 If You Have Problems
5 Storage and Stability
5 Materials Needed but Not Supplied

INTRODUCTION
6 Background
7 About This Assay

PRE-ASSAY PREPARATION
8 Reagent Preparation
9 Sample Preparation

ASSAY PROTOCOL
11 Plate Set Up
14 Performing the Assay

ANALYSIS
16 Calculations
18 Performance Characteristics

RESOURCES
19 Troubleshooting
20 References
21 Plate Template
22 Notes
23 Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
400083	ALP Assay Buffer	1 vial/30 ml	4°C
400112	ALP Substrate	1 vial/10 tablets	4°C
400113	ALP Positive Control	1 vial/10 µl	4°C
400114	ALP Stop Solution	1 vial/15 ml	4°C
400014	96-Well Solid Plate (Colorimetric Assay)	5 plates	RT
400012	96-Well Cover Sheet	5 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3640

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 405 nm
2. Adjustable pipettes and a multichannel or repeating pipettor
3. A source of pure water; glass-distilled water or HPLC-grade water is acceptable *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Alkaline phosphatase (ALP) is a ubiquitously expressed enzyme found on the cell surface that catalyzes the hydrolysis of phosphate esters at basic pH to release inorganic phosphate, which is utilized in bone mineralization, glycolysis, and oxidative phosphorylation.[1-4] There are at least four isozymes of ALP with three expressed in a tissue-specific manner in the intestine, placenta, and germ cells, and a tissue non-specific ALP (TNSALP) isozyme expressed in the liver, bone, and kidneys.[1]

Serum levels of ALP are disrupted in certain diseases and have been used as a biomarker for liver and bone diseases.[1] Baseline total ALP serum levels are typically higher in pregnant women and 3-fold higher or greater in growing children, while bone-specific ALP levels are higher in adults aged 55 years or older.[5,6] Elevated serum levels of ALPs are associated with bone diseases, such as Paget's disease, liver disease, and hyperparathyroidism, among others.[7-9] Decreased serum levels of ALP are associated with hypothyroidism, contraceptive use in women, and achondroplasia in children.[1] Levels can also be decreased or increased following certain severe acute illnesses in children.[5] TNSALP deficiency, an inborn error of pyridoxal-5'-phosphate metabolism, leads to hypophosphatasia, a disorder characterized by hypomineralization of bones and teeth, rickets, and, in severe cases, epileptic seizures and death in infancy.[10] ALP isozyme overexpression and elevated plasma levels are associated with, and used as biomarkers for, certain cancers.

About This Assay

Cayman's Alkaline Phosphatase Colorimetric Activity Assay Kit provides a convenient method of measuring ALP activity in serum, plasma, tissue samples, and cell lysates with a limit of detection of 0.5 U/L. Measurement of ALP activity is carried out by monitoring the dephosphorylation of the chromogenic substrate *p*-nitrophenyl phosphate (*p*NPP). In the first step, ALP dephosphorylates *p*NPP generating *p*-nitrophenol. In the second step, the phenolic hydroxyl group is deprotonated under alkaline conditions resulting in *p*-nitrophenolate, which yields an intense yellow color that can be measured using absorbance at 405 nm (see Figure 1). Under circumstances in which ALP activity is rate limiting, the increase in absorbance at 405 nm is directly proportional to ALP activity in the sample.

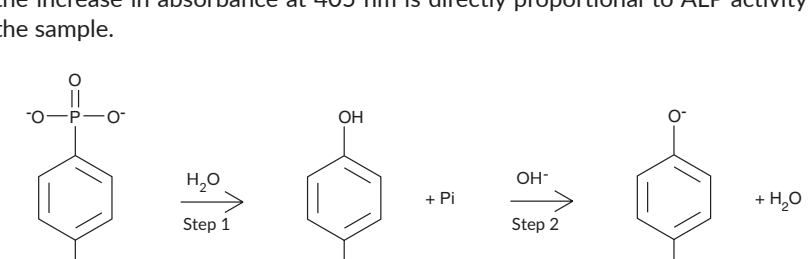

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. ALP Assay Buffer

This vial contains 30 ml of ALP Assay Buffer (Item No. 400083), and it is ready to use as supplied.

2. ALP Substrate

This vial contains *p*NPP tablets as the ALP Substrate (Item No. 400112). Dissolve two tablets in 2.7 ml ALP Assay Buffer. This is a sufficient volume of substrate to assay one 96-well plate. *NOTE: To prevent contaminating the tablets, avoid touching the tablets with bare hands.* Protect ALP substrate solution from light. It will be stable for four hours at room temperature.

3. ALP Positive Control

This vial contains 10 µl of bovine ALP as a positive control. Mix 4 µl of ALP Positive Control (Item No. 400113) with 396 µl ALP Assay Buffer to yield an ALP Positive Control (100X) stock solution. Further dilute the ALP Positive Control (100X) stock solution by mixing 4 µl of ALP Positive Control (100X) with 396 µl ALP Assay Buffer to yield an ALP Positive Control (1X) working solution. The ALP Positive Control (1X) working solution will be stable for four hours at room temperature. Both diluted ALP Positive Control enzyme stocks will be stable at 4°C for at least 72 hours.

4. ALP Stop Solution

This vial contains a solution of 2 M sodium hydroxide (NaOH). Mix 2.5 ml of ALP Stop Solution (Item No. 400114) with 7.5 ml of pure water for a final concentration of 500 mM. The diluted ALP Stop Solution is stable for at least one month when stored at room temperature.

Sample Preparation

Serum

Typically, normal human serum has ALP concentrations in the range of 7-127 U/L.[11]

1. Collect blood without using an anticoagulant such as heparin or citrate.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, store at -80°C. Avoid repeated freeze/thaw cycles.
4. Serum does not need to be diluted prior to use in the assay.

Plasma

Typically, normal human plasma has ALP concentrations similar to human serum.[12]

1. Collect blood using an anticoagulant, such as heparin or citrate. *NOTE: Chelating anticoagulants cannot be used.*
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, store at -80°C. Avoid repeated freeze/thaw cycles.
3. Plasma does not need to be diluted prior to use in the assay.

Tissue Homogenate

1. Prior to dissection, rinse the tissue with PBS, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue in 5-10 ml of cold buffer (*e.g.*, 100 mM Tris-HCl, pH 7.8) per gram of tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, store the sample at -20°C.

Cell Lysate

1. Collect cells (~5 x 10^6) by centrifugation (*e.g.*, 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, use a rubber policeman instead of proteolytic enzymes.
2. Homogenize the cell pellet in 0.5-1 ml cold buffer (*e.g.*, 100 mM Tris-HCl, pH 7.5).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant and store on ice. If not assaying on the same day, store the sample at -80°C.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is recommended that two wells be designated as background wells to substract the background absorbance and two wells be designated for the ALP Positive Control. It is suggested that each sample be assayed in at least duplicate and that the contents of each well are recorded on the template sheet provided on page 21. A typical layout of samples to be measured in duplicate is provided below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	B	B	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
B	+	+	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
C	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
D	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
E	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
F	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
G	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	S45	S45
H	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38	S46	S46

B = Background
+ = Positive Control Wells
S1-S46 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 150 µl in all the wells.
- Use the diluted assay buffer in the assay.
- All reagents must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in at least duplicate, but it is the user's discretion to do so.
- Each assay plate can accommodate 30 samples assayed in triplicate or 46 samples assayed in duplicate. With five assay plates provided, the kit can accommodate up to 150 samples in triplicate or 230 samples in duplicate.
- The assay is performed at 37°C.
- Monitor the absorbance at 405 nm.

Performing the Assay

1. **Background Wells:** add 30 µl of ALP Assay Buffer to the designated wells on the plate.
2. **Positive Control Wells:** add 10 µl of ALP Assay Buffer and 20 µl of ALP Positive Control to the designated wells on the plate (see Sample plate format, Figure 2, page 11).
3. **Sample Wells:** add 10 µl of ALP Assay Buffer and 20 µl of sample to the designated wells on the plate.
4. Quickly initiate the reactions by adding 20 µl ALP Substrate to all of the wells being used.

Well	ALP Assay Buffer	ALP Positive Control	Sample
Background	30 µl	-	-
Positive Control	10 µl	20 µl	-
Sample	10 µl	-	20 µl

Table 1. Pipetting summary

5. Cover the plate and incubate at 37°C for 20 minutes.
6. Remove the plate cover and quickly stop the reactions by adding 100 µl of ALP Stop Solution to all of the wells being used.
7. Measure the absorbance at 405 nm.

ANALYSIS

Calculations

1. Calculate the average absorbance for the background wells, positive control wells, and each sample.
2. Subtract the average absorbance of the background wells from all samples and the positive control. This is the corrected absorbance used in the equation below.
3. Use the following formula to calculate the ALP activity. One unit is defined as the amount of enzyme required to convert 1.0 µmol of *p*NPP to *p*-nitrophenol per minute at 37 °C.

$$\text{ALP Activity (U/L)} = \frac{A_{405} \times 0.15 \text{ ml}}{8.34 \text{ mM}^{-1} \times 20 \text{ min.} \times 0.00002 \text{ L}}$$

*The actual extinction coefficient of *p*-nitrophenol is 18.45 $mM^{-1}cm^{-1}$. This value has been adjusted for the pathlength of the solution in the well (0.452 cm).

Spike and Recovery

Assay buffer, mouse liver tissue extract, and human serum were spiked with ALP and analyzed using the ALP Colorimetric Activity Assay Kit. Percent recovery of the ALP in plasma and tissue extract was calculated by comparison with the measured activity of the same spikes in buffer. The results are shown below. The error bars represent standard deviations between multiple measurements of ALP activity.

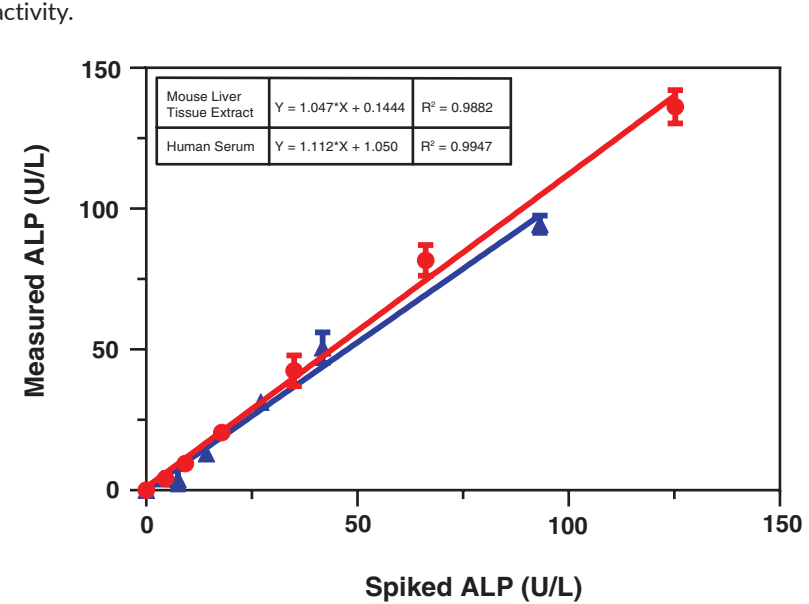

Figure 3. Spike and recovery in mouse liver tissue extract and human serum

Performance Characteristics

Range of the Assay:

The linear range of this assay is 0.5-130 U/L.

Precision:

When a series of 48 measurements of the positive control and 24 measurements of two plasma samples were performed on the same day under the same experimental conditions, the intra-assay coefficients of variation were 5.0, 4.5, and 2.4%, respectively. When a series of measurements of the positive control and two plasma samples were performed in 8 separate assays under the same experimental conditions, the inter-assay coefficients of variation were 7.2, 4.5, and 6.1%, respectively.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
Low absorbance of both samples and control	A. Plate was not incubated at 37°C B. Cold buffer used in assay	A. Re-assay and incubate plate at 37°C B. Re-assay using reagents equilibrated to room temperature
Absorbance over the limit of detection	ALP in the sample is beyond the linear range of the assay	Dilute sample in ALP Assay Buffer and re-assay

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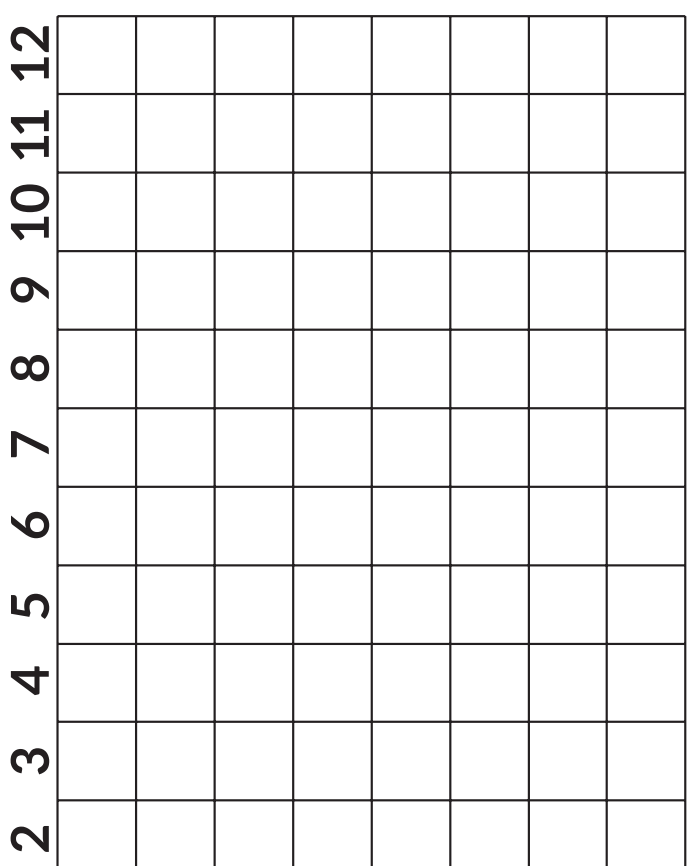

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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