ab83371
Alkaline Phosphatase Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of alkaline phosphatase in various samples.

View kit datasheet: www.abcam.com/ab83371
(use www.abcam.cn/ab83371 for China, or www.abcam.co.jp/ab83371 for Japan)

This product is for research use only and is not intended for diagnostic use.

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.

Version 9 Last Updated 7 June 2023

Table of Contents

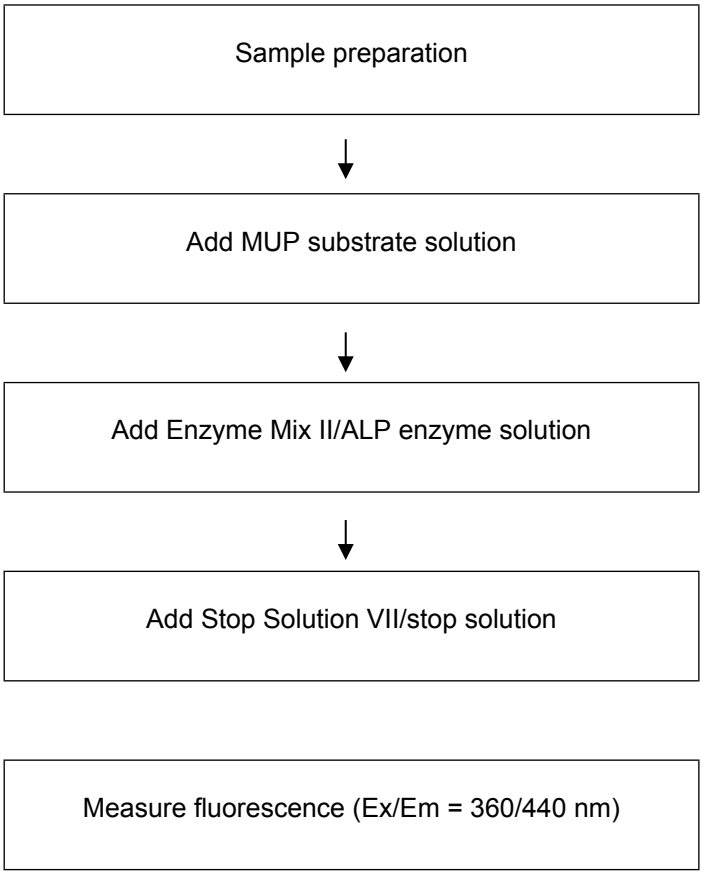
1. BACKGROUND

Alkaline Phosphatase Assay Kit (fluorometric) (ab83371), ALP cleaves the phosphate group of the non-fluorescent 4-methylumbelliferyl phosphate disodium salt (MUP) substrate resulting in an intense fluorescent signal (Ex/Em = 360 nm / 440 nm).

This kit is an ultra-sensitive, simple, direct and HTS-ready assay designed to measure ALP activity in serum and bio-samples with detection sensitivity ~1 µU, more sensitive than colorimetric assays.

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in alkaline buffer and produces an organic radical and inorganic phosphate. The change in alkaline phosphatase level and activity is associated with a lot of diseases in the liver and bones. Alkaline phosphatase is also a popular enzyme conjugated to secondary antibody in ELISA.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC 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. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
ALP Assay Buffer I/ALP Assay Buffer	100 mL	-20°C	-20°C
MUP Substrate (5 mM)	1 vial	-20°C	-20°C
Enzyme Mix II/ALP Enzyme	1 vial	-20°C	4°C
Stop Solution VII/Stop Solution	25 mL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- PBS
- Fluorescent microplate reader – equipped with filter for Ex/Em = 360/440 nm
- 96 well plate: black plates (clear bottoms) for fluorometric assay
- Microcentrifuge
- Pipettes and pipette tips
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **ALP Assay Buffer I/ALP Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **MUP Substrate (MUP Standard):**

Dissolve MUP substrate in 1.2 mL ALP Assay Buffer I/Assay Buffer to generate 5 mM MUP substrate solution (also used as MUP standard). Aliquot substrate so that you have enough to perform the desired number of assays. Store at -20°C. Use within 2 months.

9.3 **Enzyme Mix II/ALP Enzyme Solution:**

Reconstitute Enzyme Mix II/ALP Enzyme with 1 mL ALP Assay Buffer I/Assay Buffer. The reconstituted enzyme is stable for up to 2 months at 4°C. Aliquot enzyme so that you have enough to perform the desired number of assays. DO NOT FREEZE! Keep on ice whilst in use.

9.4 **Stop Solution VII/Stop Solution:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 Prepare a 50 µM MUP standard by diluting 5 µL of the 5 mM MUP substrate (standard) with 495 µL ALP Assay Buffer I/Assay Buffer.

10.2 Using 50 µM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	ALP Assay Buffer I/Assay Buffer (µL)	Final volume standard in well (µL)	End [MUP] in well
1	0	360	120	0 nmol/well
2	6	354	120	0.1 nmol/well
3	12	348	120	0.2 nmol/well
4	18	342	120	0.3 nmol/well
5	24	336	120	0.4 nmol/well
6	30	330	120	0.5 nmol/well

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

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^5cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ALP Assay Buffer I/Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge 3 minutes at 4°C at 13,000 x g using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg.

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ALP Assay Buffer I/Assay Buffer.

11.2.4 Homogenize using a Dounce homogenizer (10-50 passes) on ice.

11.2.5 Centrifuge 3 minutes at 4°C at 13,000 x g using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.3 **Plasma and Serum Samples:**

Plasma and serum samples can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/10 – 1/100).

11.4 **Other biological samples:**

Urine, semen, saliva samples and cell culture media can be tested directly by adding sample to the microplate wells.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 Set up Reaction wells:

- Standard wells = 120 µL standard dilutions.
- Sample wells = 1 – 110 µL samples (adjust volume to 110 µL/well with ALP Assay Buffer I/Assay Buffer).
- (Optional) Sample background control = 1 – 110 µL samples (adjust volume to 110 µL/well with ALP Assay Buffer I/Assay Buffer).
- Background wells = 110 µL ALP Assay Buffer I/ALP Assay Buffer.

12.2 MUP Reaction Mix:

Prepare Reaction Mix for each reaction:

Component	Reaction Mix (µL)
MUP substrate (5 mM)	**2**
ALP Assay Buffer I/Assay Buffer	**18**

Mix enough reagents for the number of assays (samples and background) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + background (buffer only) +1)

12.3 Add the following to the samples shown below, and mix well:

Sample Description	MUP Reaction Mix (µL)	Stop Solution VII/Stop Solution
Sample (test)	**20**	**0**
Sample (test) background control	**0**	**20**
Background control (ALP Assay Buffer I/assay buffer)	**20**	**0**

12.4 To the standard curve wells, add 10 µL of Enzyme Mix II/ALP enzyme.

NOTE: *The Enzyme Mix II/ALP enzyme will convert MUP substrate to equal amount of fluorescent 4-Methylumbelliferone (4-MU).*

12.5 Incubate for 30 minutes at 25ºC protected from light.

12.6 Add 20 µL Stop Solution VII/Stop Solution to sample, standard and background well.

NOTE: *Do not add Stop Solution VII/Stop Solution to sample background wells (as it has already been added in step 12.3).*

12.7 Gently shake the plate.

12.8 Measure output on a microplate reader.

- Fluorometric assay: measure Ex/Em = 360/440 nm.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the appropriate background from all standard and sample readings if applicable.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of 4-MU.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Apply sample readings to the standard curve to get the amount of 4-MU generated by ALP sample

13.7 Activity (mU/mL) of alkaline phosphatase in the test samples is calculated as:

$$ALP\ activity = B/V/T$$

Where:

B = amount of 4-MU generated by samples (in nmol).

V = volume of sample added in the assay well (in mL).

T = reaction time (in minutes).

Unit Definition: The amount of enzyme causing the hydrolysis of 1 µmol of MUP per minute at pH 10.0 and 25°C (glycine buffer).

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

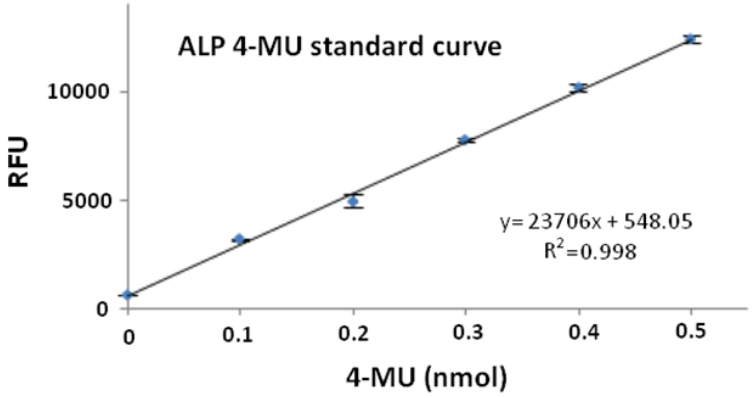


Figure 1: Typical alkaline phosphatase 4-MU standard calibration curve using fluorometric reading.

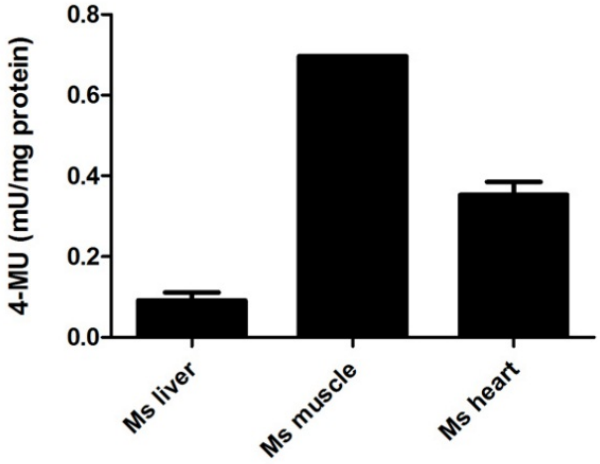


Figure 2: 4-MU measured in mouse tissue lysates showing quantity (mU) per mg protein of tested sample.

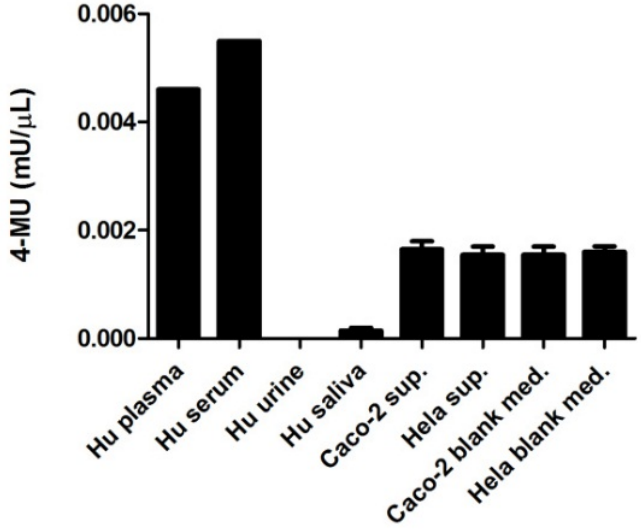


Figure 3: 4-methylumbelliferyl measured in biological fluids showing quantity (mU) per microliter of tested sample.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare MUP standard (substrate) solution and Enzyme Mix II/ALP Enzyme. Thaw ALP Assay Buffer I/ALP Assay Buffer, Stop Solution VII/Stop Solution (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Prepare MUP Reaction Mix (Number samples + standards + 1)

Component	Fluorometric Reaction Mix (µL)
MUP substrate (5 mM)	**2**
ALP Assay Buffer I/Assay Buffer	**18**

- Set up plate for standard (120 µL), samples (110 µL), background (110 µL) and sample background (110 µL).
- Add 20 µL MUP Reaction mix to each sample and background control (buffer only) well.
- (Optional) Add 20 µL of Stop Solution VII/Stop Solution to each sample background well.
- Add 10 µL of Enzyme Mix II/ALP enzyme to each standard well.
- Incubate 30 min 25ºC protected from light.
- Add 20 µL Stop Solution VII/Stop Solution to sample, standard and background wells.
- Measure output on a microplate reader at Ex/Em = 360/440 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

We would like to use ab65621 with protein samples isolated that contain protease inhibitor vs samples that do not contain protease inhibitor. Are there any other components that we should be aware of that could affect the performance of this kit when one uses protease inhibitors?

The only chemicals you need to be wary of are EDTA, oxalate, fluoride, and citrate.

Which protease inhibitor is recommended for use in this alkaline phosphastase assay kit?

There is no recommended protease inhibitor for this assay kit. You can use our ab65621 cocktail.

We wish to measure samples at different time points. So, during the sample preparation, once ALP Assay Buffer I/ALP assay buffer is added to the cell samples, can I then freeze these samples to -20ºC?

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

How do we normalize our final readings?

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

When the enzyme is re-suspended and stored at 4ºC what is the reduction of viability of this when used at time points after the two months recommended, for example three or four months later?

We have not checked the enzyme stability statistics at 4°C for longer than 2 months. There is a fair chance that it will be stable for slightly

longer, but we do not know exactly how long and then after that time how much of the stability/efficiency would be lost.

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure

- Tartrate.
- EDTA.
- Oxalate.
- Citrate.
- Fluoride.

19. **NOTES**

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp