Technical Bulletin

Alkaline Phosphatase Activity Fluorometric Assay Kit

Catalog Number MAK411

Product Description

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 several diseases in the liver and bones. Alkaline phosphatase is also a common enzyme conjugated to secondary antibodies for ELISA.

In the Alkaline Phosphatase Activity Fluorometric Assay Kit, ALP cleaves the phosphate group of the non-fluorescent 4-Methylumbelliferyl phosphate disodium salt (MUP) substrate resulting in an intense fluorescent signal (λ_{Ex} = 360 nm/ λ_{Em} = 440 nm). The assay is very sensitive, simple, direct, and suitable for high-throughput applications. The method is designed to measure ALP activity in serum and other biological samples with a detection sensitivity of ~1 μU, more sensitive than colorimetric assays. The kit is suitable for both research and drug discovery.

The kit is suitable for the measurement of alkaline phosphatase activity in serum, plasma, urine, semen, cell culture media, cells and tissue.

Components

The kit is sufficient for 500 fluorometric assays in 96-well plates.

- ALP Assay Buffer 100 mL
  Catalog Number MAK411A
- MUP Substrate 1 vial
  Catalog Number MAK411B
- ALP Enzyme 1 vial
  Catalog Number MAK411C
- Stop Solution 25 mL
  Catalog Number MAK411D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories(e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Microcentrifuge capable of RCF ≥13,000 × *g*

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions.

Briefly centrifuge small vials prior to opening.

ALP Assay Buffer: Ready to use. Allow ALP Assay Buffer to warm to room temperature prior to use.

MUP Substrate Solution: Reconstitute MUP Substrate with 1.2 mL of ALP Assay Buffer to generate a 5 mM MUP substrate solution. The MUP substrate solution is stable for two months at -20 °C after reconstitution.

ALP Enzyme Solution: Reconstitute ALP Enzyme with 1 mL of ALP Assay Buffer. The reconstituted enzyme is stable for up to two months at 2-8 °C. **DO NOT FREEZE after reconstitution!**

Ensure that the ALP Assay Buffer is at room temperature prior to use. Keep samples and ALP Enzyme Solution on ice during the assay.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Note: Inhibitors of ALP, like tartrate, fluoride, EDTA, oxalate, and citrate, should be avoided in sample preparation.

Serum, Plasma, Urine, Semen, and Cell Culture Media

Serum, plasma, urine, semen, and cell culture media can be assayed directly.

Cells and Tissue

Homogenize cells (1×10^5 cells) or tissue (~10 mg) in 100 µL of ALP Assay Buffer. Centrifuge to remove insoluble material at 13,000 × *g* for 3 minutes and collect the supernatant. Add test samples directly into 96-well plate.

For All Samples

Add 1-20 µL of samples into a 96-well plate that will be designated as Sample(s). It is recommended to use 3-5 different volumes of each sample in separate wells to ensure the readings are within the Standard Curve range.

Adjust the total volume of each Sample (S) well to 110 µL with ALP Assay Buffer.

Sample Background Control (SBC)

In order to avoid interference of components in the sample, prepare a Sample Background Control (SBC) for each Sample (S).

1. Add the same volume of samples into duplicate wells with one well designated as Sample (S) and one as Sample Background Control (SBC).
2. Adjust the total volume of each Sample (S) and Sample Background Control (SBC) well to 110 µL with ALP Assay Buffer.
3. Add 20 µL of Stop Solution to the Sample Background Control (SBC) well to terminate ALP activity. Mix well.

Sample and Sample Background Control Reaction

1. Prepare a 0.5 mM substrate solution by diluting the 5 mM MUP substrate solution 1:10 with ALP Assay Buffer. Prepare 20 µL for each Sample (S) and Sample Background Control (SBC) well.
2. Add 20 µL of the 0.5 mM MUP substrate solution to each well containing Sample (S) and Sample Background Control (SBC). Mix well.
3. Incubate the reaction for 30 minutes (or longer if ALP activity in sample is low) at 25 °C, protected from light.
4. Continue with Step 4 of Standard Curve Preparation (Stopping reaction).

Standard Curve Preparation

1. Prepare a 50 µM MUP Standard by diluting 10 µL of the 5 mM MUP Substrate Solution with 990 µL of ALP Assay Buffer. Prepare MUP Standards according to Table 1. Mix well.

Table 1.
Preparation of MUP Standards

Well	50 µM MUP Standard	ALP Assay Buffer	MUP (nmol/ well)
1	0 µL	120 µL	0
2	2 µL	118 µL	0.1
3	4 µL	116 µL	0.2
4	6 µL	114 µL	0.3
5	8 µL	112 µL	0.4
6	10 µL	110 µL	0.5

2. Add 10 µL of ALP Enzyme Solution to each well containing the MUP Standards. Mix well.
3. Incubate the reaction for 30 minutes at 25 °C, protected from light. The ALP enzyme will convert MUP substrate to an equal amount of fluorescent 4-Methylumbelliferone (4-MU).
4. Stop reactions by adding 20 µL of Stop Solution into each Standard and Sample (S) reaction wells (**Do not add** to Sample Background Control (SBC) wells since Stop Solution was previously added). Gently shake the plate to mix.

Measurement

Measure the fluorescence (RFU) of all wells at λ_{Ex} = 360 nm/λ_{Em} = 440 nm at room temperature in end point mode.

Results

1. Subtract the 0 Standard RFU reading from all Standard readings.
2. Plot the 4-MU standard Curve.
3. Correct for background by subtracting the RFU reading for each Sample Background Control (SBC) from the RFU reading for its corresponding Sample (S).
4. Apply the corrected Sample readings to the Standard Curve to get the amount of 4-MU generated.
5. Calculate the ALP activity of the Sample using the following equation:

   ALP Activity (mU/mL) = A/V/T

where:

A = Amount of 4-MU generated by Sample (in nmol)

V = Volume of sample added in the assay well (in mL) (1 mL = 1000 µL)

T = Reaction time (in minutes). The standard protocol has a reaction time of 30 minutes.

Unit Definition: One unit of alkaline phosphatase (ALP) is the amount of enzyme that hydrolyzes 1 µmol of MUP per minute at pH 10.0 and 25 °C (glycine buffer).

Figure 1.
Typical 4-MU Standard Curve

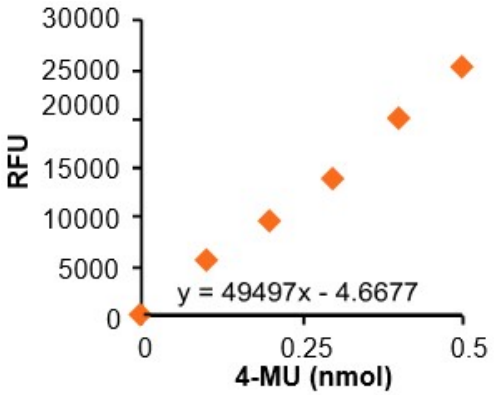


Figure 2.
Measurement of ALP activity in fresh medium (80 µL, without culturing), 3-day old HeLa cell cultured medium (80 µL) and human serum (80 µL, 1:10 diluted).

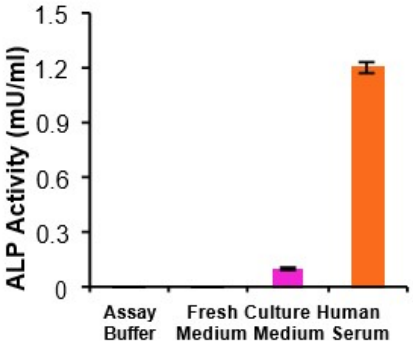
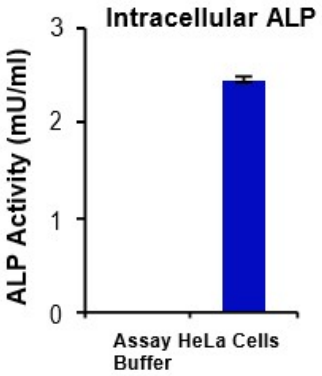


Figure 3.
Measurement of ALP activity in HeLa cells: 1×10^4 HeLa Cells were homogenized in 200 µL of Assay Buffer and diluted 1:10 in Assay Buffer. 80 µL of diluted supernatant was used to measure intracellular ALP activity. Assays were performed according to the kit protocol.



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.