Version 2a Last updated 16 September 2025

ab233466 Alkaline Phosphatase Assay Kit (Luminometric)

For the measurement of Alkaline Phosphatase activity in solutions and cells.

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

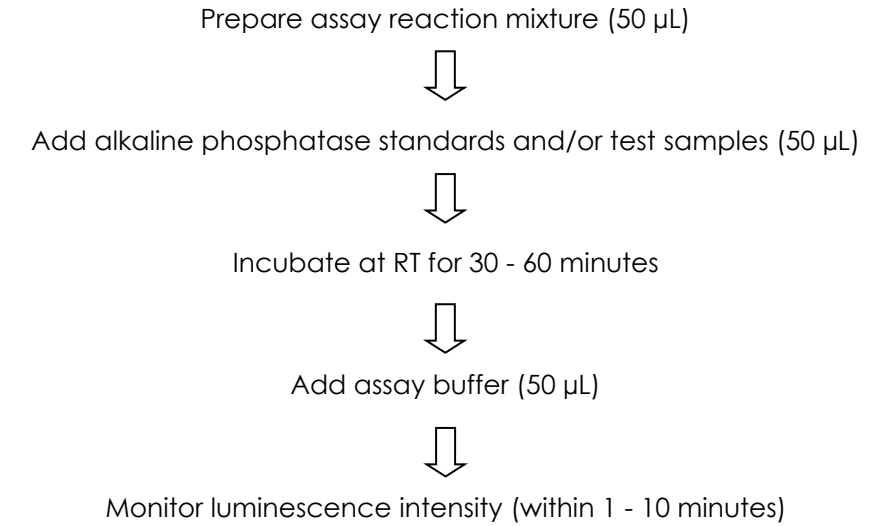
Table of Contents

1. Overview

Alkaline Phosphatase Assay Kit (ab233466) uses D-luciferin phosphate as the luminogenic phosphatase substrate to quantify alkaline phosphatase activity in solutions and in cells.

D-luciferin phosphate is not recognized by luciferase until its phosphate group is removed to give luciferin. This kit provides all the essential components with an optimized assay protocol which is compatible with HTS liquid-handling instruments. The assay can be readily performed in a 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

The high sensitivity makes the kit ideal for the assays that require low detection limit.



2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Phosphatase Substrate	1 vial	-20°C	-20°C
Reaction Buffer	5 mL	-20°C	-20°C
Alkaline Phosphatase Standard	1 vial	-20°C	-20°C
Assay Buffer	5 mL	-20°C	-20°C

3. Materials Required, Not Supplied

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

- Luminescence microplate reader
- 96/384 well solid white microplate.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Phosphatase Substrate

Lyophilized. To make the assay reaction mixture, mix the whole content of Phosphatase Substrate with Reaction Buffer and keep from light

5.2 Reaction Buffer

Ready to use as supplied.

5.3 Alkaline Phosphatase Standard

Add 100 µL of distilled H_2O with 0.1% BSA into the vial of Alkaline Phosphatase Standard to generate a 100 units/mL Alkaline Phosphatase standard solution.

Δ Note: The alkaline phosphatase standard solution is not stable. Unused solution should be aliquoted and stored at -20°C. Avoid repeated freeze and thaw cycles.

5.4 Assay Buffer

Ready to use as supplied. Precipitation of the assay buffer will not interfere with the assay.

6. Standard Preparation

– Always prepare a fresh set of standards for every use.

1. Add 10 µL of 100 units/mL Alkaline Phosphatase standard solution (from Step 5.3) into 990 µL of H_2O- 0.1% BSA to generate a 1,000 mU/mL Alkaline Phosphatase standard solution.
2. Add serially diluted Alkaline Phosphatase standards and/or Alkaline Phosphatase containing test samples into a solid white 96-well microplate as described below in Tables 1 and 2.
   Δ Note 1: Prepare cells or tissue samples as desired.
   Δ Note 2: Unused serial dilutions of alkaline phosphatase standard should be discarded.

BL	BL	TS	TS	…	…						
AS1	AS1	…	…	…	…						
AS2	AS2										
AS3	AS3										
AS4	AS4										
AS5	AS5										
AS6	AS6										
AS7	AS7										

Table 1. Layout of alkaline phosphatase standards and test samples in a solid white 96-well microplate: AS = Alkaline Phosphatase standards; BL = Blank Control; TS = Test samples.

Alkaline Phosphatase standards	Blank control	Test sample
* 50 µL	H_2O–0.1% BSA: 50 µL	50 µL

Table 2. Reagent composition for each well.

* Add serially diluted Alkaline Phosphatase standards from 10 to 0.01 mU/mL into wells from AS1 to AS7 in duplicate.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 Run Alkaline Phosphatase assay in supernatants:

1. To make the assay reaction mixture, mix the whole content of Phosphatase Substrate with Reaction Buffer and protect from light.
2. Add 50 µL of assay reaction mixture (from Step 1) into each well of alkaline phosphatase standard, blank control, and test samples to make the total alkaline phosphatase assay volume of 100 µL/well.
   Δ Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.
3. Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.
4. Add 50 µL of Assay Buffer into each well of alkaline phosphatase standard, blank control, and test samples with assay reaction mixture to make the total alkaline phosphatase assay volume of 150 µL/well.
   Δ Note: For a 384-well plate, add 25 µL of Assay Buffer into each well.
5. Read immediately after the addition of Assay Buffer, protected from light.
6. Monitor the luminescence increase with a standard luminescence plate reader.

7.2 Run Alkaline Phosphatase assay in cells:

1. To make the assay reaction mixture, mix the whole content of Phosphatase Substrate with Reaction Buffer and keep from light.
2. Treat the cells as desired.
3. Remove the growth medium completely from the cell plate.
   Δ Note: It is important to remove the growth medium completely from the cell plate due to the interference of the growth medium with the phosphatase substrate.
4. Make a 1:1 dilution of the 5 mL assay reaction mixture (from Step 1) with 5 mL distilled H_2O.
5. Add 100 µL (96-well plate) or 50 µL (384-well plate) of 1:1 diluted assay reaction mixture into the cell wells.
6. Incubate the reaction at the desired temperature for 30 to 60 minutes, protected from light.
7. Add 50 µL (96-well plate) or 25 µL (384-well plate) of Assay Buffer into the cell wells containing assay reaction mixture.
8. Read immediately after the addition of Assay Buffer, protected from light.
9. Monitor the luminescence increase with a standard luminescence plate reader.

8. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank from all standards, controls and sample readings. This is the corrected absorbance.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of Alkaline Phosphatase.
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).
6. Apply the corrected sample OD reading to the standard curve to get Alkaline Phosphatase amount in the sample wells.
7. Concentration of Alkaline Phosphatase in the test samples is calculated as:

$$Alkaline\ Phosphatase\ concentration = \frac{B}{V} * D$$

Where:

B = amount of Alkaline Phosphatase in the sample well calculated from standard curve in mU/mL.

V = sample volume added in the sample wells.

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

Δ Note: The luminescence background increases with time due to spontaneous hydrolysis, thus it is important to subtract the luminescence intensity value of the blank wells for each data point.

9. Typical Data

Data provided **for demonstration purposes only**.

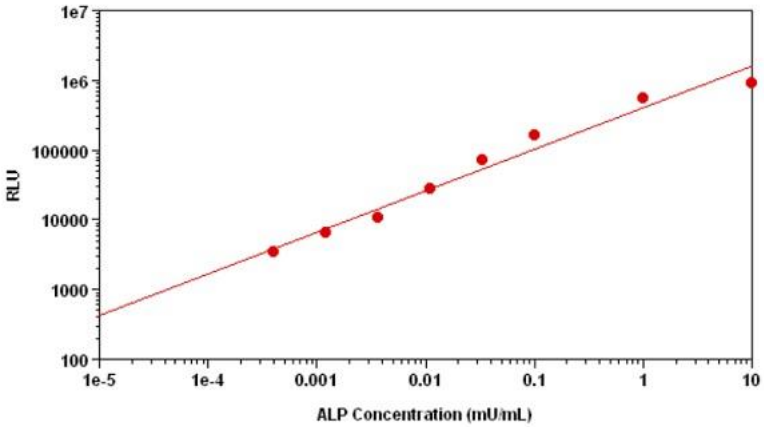


Figure 1. Alkaline phosphatase dose response was measured with Alkline Phosphatase Assay Kit (Luminometric) (ab233466) in a white 96-well plate using a NovoStar microplate reader (BMG Labtech). As low as 0.001 mU/mL alkaline phosphatase can be detected with 20 minutes incubation (n=3).

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)