ab138887

Alkaline Phosphatase Assay Kit (Fluorometric - Near Infrared)

Instructions for Use

For detecting Alkaline Phosphatase activity in solutions and cell extracts

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

Version 2a Last updated 4 June 2020

Table of Contents

1. Introduction

Alkaline phosphatase is widely used in various biological assays (in particular, immunoassays) and ELISA-based diagnostics. Our Alkaline Phosphatase Assay kit (Fluorometric-Near Infrared) uses our SunRed Dye based substrate. The weakly fluorescent SunRed Dye phosphate is sensitive to phosphatase-induced hydrolysis, giving the SunRed Dye fluorophore that possesses intense red fluorescence. Upon phosphatase-induced hydrolysis, the SunRed Dye phosphate solution has its absorption blue-shifted more than 100 nm. The maximum absorption of SunRed Dye fluorophore at 633 nm makes this substrate an ideal NIR probe that can be readily detected with many fluorescence instrument systems often equipped with Cy5 settings. Based on the near infrared fluorescence of SunRed Dye fluorophore, the signal can be easily read by a fluorescence microplate reader at Ex/Em = ~630/660 nm.

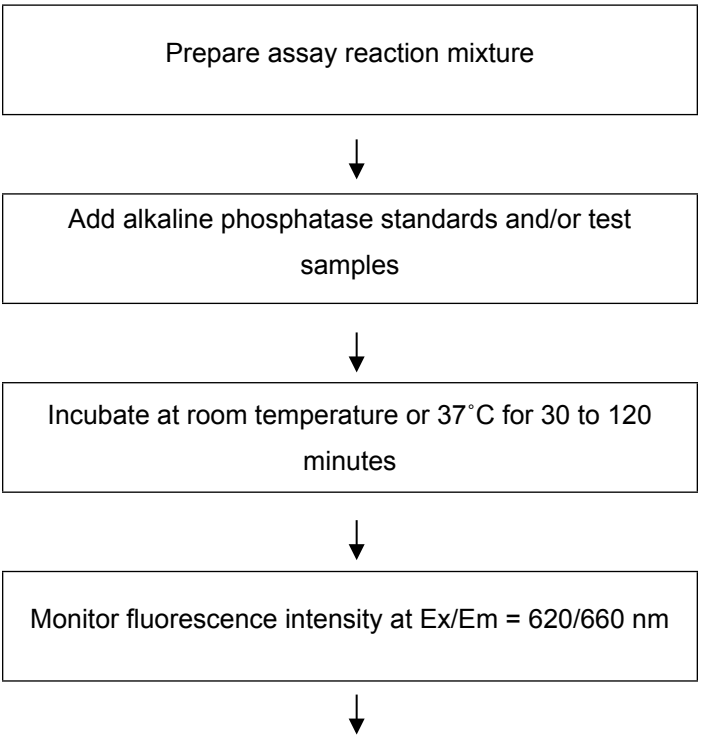
ab138887 is designed to accommodate high throughput screening of protein phosphatase inhibitors due to its low interference from biological samples. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

Kit Key Features

- **Optimized:** Optimized conditions for detecting alkaline phosphatase activity.
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time. No wash is required.
- **Non-Radioactive:** No special requirements for waste treatment.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: SunRed Dye Substrate (light sensitive)	1 vial
Component B: Assay Buffer	1 x 25 mL
Component C: Alkaline Phosphatase Standard (lyophilized powder)	1 x 10 U

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Additional Materials Required

- 96 or 384-well solid, black microplates: Tissue culture microplates with white wall and clear bottom
- Fluorescence microplate reader
- Distilled H_2O with 0.1 % BSA

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Prepare SunRed Dye Substrate stock solution (250X):

Add 100 µL of double sterile H_2O into the vial of SunRed Dye Substrate (Component A). The stock solution should be used promptly. Any remaining solution needs to be aliquoted and refrozen at -20 °C.

Note: Avoid repeated freeze and thaw cycles.

B. Prepare Assay Reaction Mixture

Prepare assay reaction mixture according to the following table, kept from light.

Components	Volume
250X SunRed Dye Substrate stock solution (Step A)	20 µL
Assay Buffer (Component B)	5 mL
Total volume	5 mL

Table 1. Assay reaction mixture for one 96-well plate

Note: Prepare fresh reaction mixture for each experiment.

C. Prepare serially diluted alkaline phosphatase standards (0 to 100 mU/ml):

1. Add 100 µL of distilled H_2O with 0.1% BSA (H_2O-0.1% BSA) to alkaline phosphatase standard (Component C, 10 units) to generate a 100 units/mL alkaline phosphatase standard solution.

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

2. Add 10 µL of 100 units/mL alkaline phosphatase standard solution (from Step 1) to 990 µL of H_2O-0.1 % BSA to generate a 1,000 mU/mL alkaline phosphatase standard solution.

3. Take 100 µL of 1,000 mU/mL alkaline phosphatase standard solution (from Step 2) to perform 1:10 and then 1:3 serial dilutions to get 100, 30, 10, 3, 1, 0.3, 0.1, and 0 mU/mL serially diluted alkaline phosphatase standards.

4. Add serially diluted alkaline phosphatase standards and/or alkaline phosphatase containing test samples into a solid black 96-well microplate as described in Tables 2 and 3.

Note 1: Prepare cells or tissue samples as desired.

Note 2: Unused serially diluted alkaline phosphatase standards should be discarded

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

Table 2. Layout of Alkaline Phosphatase standards and test samples in a solid black 96-well microplate.

Note: AS= Alkaline Phosphatase *Standards; BL=Blank Control; TS=Test Samples.*

Alkaline Phosphatase Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	H_2O-0.1% BSA: 50 µL	50 µL

Table 3. Reagent composition for each well.

**Note: Add the serially diluted alkaline phosphatase standards from 100 to 0.01 mU/mL into each well from AS1 to AS7 in duplicate.*

D. Run alkaline phosphatase assay in supernatants:

1. Add 50 µL of assay reaction mixture (from Step B) to each well of alkaline phosphatase standard, blank control, and test samples (Table 3) to make the total alkaline phosphatase assay volume of 100 µL/well.

Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.

2. Incubate the reaction for 30 to 120 minutes at the desired temperature, protected from light.

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

3. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 630±10 /660±10 nm.

E. Run alkaline phosphatase assay in cells:

1. Treat the cell as desired.

2. Remove the growth medium completely from the cell plate.

Note: It is important to remove the growth medium completely from the cell plate due to the interference of the growth medium with the SunRed Dye Substrate.

3. Make 1:1 dilution of the 5 mL assay reaction mixture (from Table 1) with 5 mL distilled H_2O.
4. Add 100 µL (96-well plate) or 50 uL (384-well plate) of 1:1 diluted assay reaction mixture (from Step 3) into each cell well (from Step 2).
5. Incubate the reaction for 30 to 60 minutes at the desired temperature, protected from light.
6. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 630 ± 10/660±10 nm.

7. Data Analysis

The fluorescence in blank wells (with equal volume of assay reaction mixture and H_2O-0.1 % BSA only) is used as a control, and is

subtracted from the values for those wells with alkaline phosphatase reactions. An alkaline phosphatase standard curve is shown in Figure 1.

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

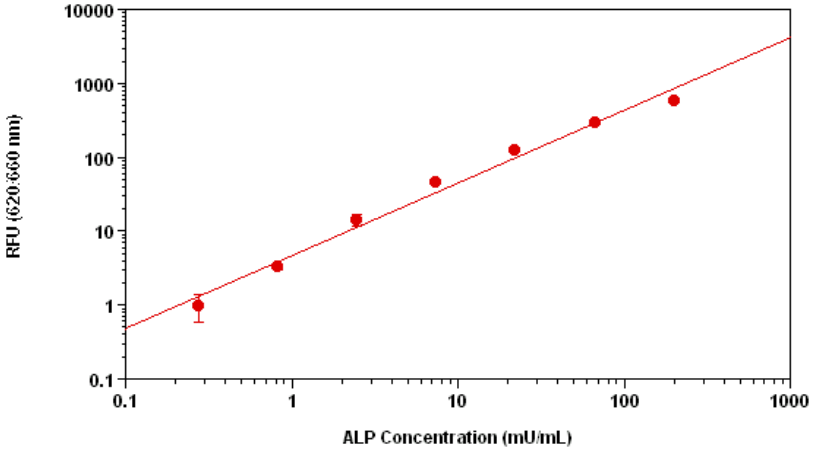


Figure 1. Alkaline phosphatase dose response was measured with the Alkaline Phosphatase Assay Kit (Fluorometric –Near Infrared) in a solid black 96-well plate using a Gemini microplate reader (Molecular Devices). As low as 0.3 mU/mL alkaline phosphatase can be detected with 60 minutes incubation (n=3).

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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