ab102508

Phosphate Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Inorganic Phosphate levels in various samples.

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

Version 6b, Last Updated 7 March 2024

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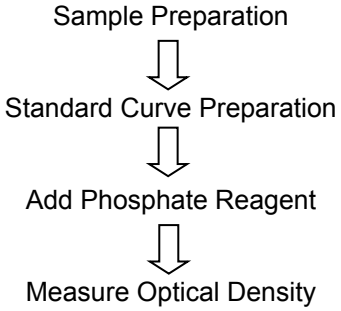
1. Overview

Inorganic phosphate (Pi) is one of the most important ions in biological systems. It functions in a variety of roles. One of the most important roles is as a molecular switch, turning enzyme activity on and off through the mediation of the various protein kinases and phosphatases in biological systems. A highly sensitive assay is desired to monitor Pi in variety samples or monitor Pi changes during kinase and phosphatase reactions.

Abcam's Phosphate Assay Kit (Fluorometric) provides a sensitive, easy, quick means of assessing phosphate over a wide range of concentrations. In the assay, inorganic phosphate reacted with sucrose to produce glucose-1-phosphate in the present of a proprietary enzyme. The glucose-1-phosphate is specifically oxidized to generate a product that reacts with the PicoProbe™ probe to generate fluorescence (Ex/Em=535/587nm).

The kit can be used to detect Pi produced through reactions involving ATPases, GTPases, 5'-nucleotidase, protein phosphatases, acid and alkaline phosphatases, and phosphorylase, etc. from a variety of samples. This assay is not affected by the presence of glucose in samples. Phosphate concentrations between 2 µM and 10 µM, with a lower detection limit of approximately 100 pmol can be determined.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Phosphate Assay Buffer	25 mL
PicoProbe™ (in DMSO)	200 µL
Converter (Lyophilized)	1 vial
Developer (Lyophilized)	1 vial
Phosphate Substrate	0.2 mL
Phosphate Standard (10 mM)	500 µL

* Store kit at -20°C, protect from light. Allow reagents to warm to room temperature and briefly centrifuge vials prior to opening. Read the entire protocol before the assay.

PicoProbe™ (in DMSO): Store at -20°C, protected from light. Use within two months.

CONVERTER, DEVELOPER: Dissolve in 220 µl Assay Buffer separately. Aliquot and store at -20°C. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

CAUTION: Phosphate contamination in samples and buffers must be carefully avoided. Laboratory detergents can contain high concentrations of phosphates and glassware must be thoroughly rinsed with distilled water to remove any phosphate bound to the glass.

1. Sample Preparation:

Add 1-50 µl test samples in a 96-well plate; bring the volume to total 50 µl/well with Assay Buffer. If using serum sample, serum***** (0.5-2 µl/well) can be directly diluted in the Assay Buffer.

We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

*** Note:** Serum contains 4-6 mM glucose, and will interfere with the result; therefore a glucose control is necessary.

2. Standard Curve Preparation:

Dilute the Phosphate Standard to 100 µM by adding 10 µl of the Phosphate Standard (10 mM) to 990 µl of Assay Buffer, mix well, then take 20 µl into 180 µl of Assay Buffer. Mix well.

Add 0, 2, 4, 6, 8, 10 µl of 100 µM standard into each well individually. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 200, 400, 600, 800, 1000 pmol/well of the Phosphate Standard.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

Assay Buffer	43 µl
PicoProbe™	1 µl
Phosphate Substrate	2 µl
Converter**	2 µl
Developer	2 µl

Add 50 µl of the Reaction Mix to each well containing the Phosphate Standard and test samples, mix well.

** **Note:** Glucose interferes with Pi the reaction. If a significant amount of glucose is in your sample, you may do a glucose control by omitting the Converter in the reaction, which will read glucose background only. The glucose background should be subtracted from Pi readings.

4. Add 50 µl of the Reaction Mix to each well containing the Phosphate Standard and test samples, mix well.

5. Incubate the reaction for 1 hours at room temperature, protect from light.

6. Measurement: Measure fluorescence at Ex/Em = 535/587 nm in a microplate reader.

5. Data Analysis

Correct background by subtracting the value derived from the zero phosphate control from all sample readings. The background reading can be significant and must be subtracted from sample readings.

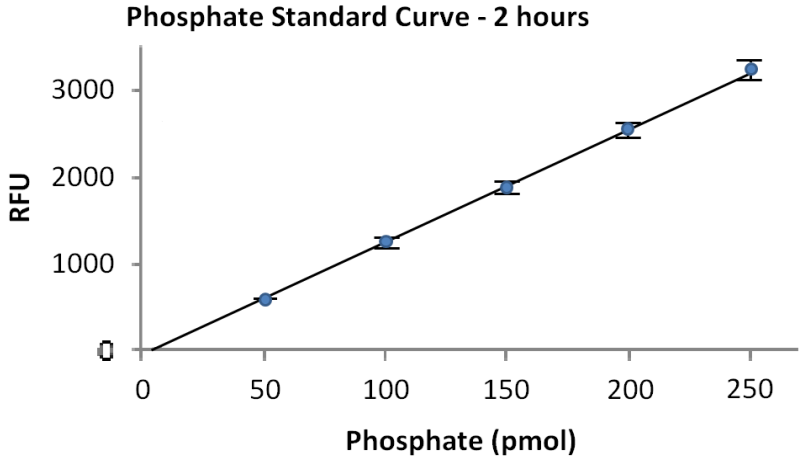
Plot the Pi Standard Curve; apply the sample readings to the standard curve.

Concentration = A / V (pmol/µl, or µM)

Where:

A is the Pi amount in the reaction from standard curve (in pmol)

V is sample volume added into the reaction well (in µl)



Phosphate Standard Curve: Performed following the kit protocol.

6. 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

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)***
	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)

Problem	Reason	Solution
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

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)