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ab273281
Phosphate Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273281
(use https://www.abcam.cn/ab273281 for china, or
https://www.abcam.co.jp/ab273281 for Japan)

For the determination of Phosphate content in serum, tissues and cells.

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

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1. Overview

Phosphate Assay Kit (Fluorometric) (ab273281) provides an easy, quick and sensitive means of assessing phosphate over a wide range of concentrations. In the assay, inorganic phosphate will react with substrate and the Probe to generate fluorescence (Ex/Em = 535/587 nm). The kit can be used to detect P_i in a variety of samples or to monitor phosphate released by an assortment of enzymes (such as ATPases, GTPases, 5'-nucleotidase, protein phosphatases, acid and alkaline phosphatases, and phosphorylase kinase). Unlike other commercially available assays, the assay is not affected by the presence of glucose in samples (Note: Glucose interferes with many other commercially available assays). This assay is highly sensitive with the detection limit of approximately 40 pmol/well.

2. Protocol Summary

Prepare samples as directed

Prepare all reagents as directed

Prepare standard curve

Add Standard, Sample and Background Control to appropriate wells and adjust volume to 50 µL

Add Reaction Mix to Standard and Sample wells. Add Background Control Mix to Background Control wells (50 µL)

Incubate plate at 37°C for 30 minutes

Measure fluorescence (Ex/Em = 535/587 nm)

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

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

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Phosphate Assay Buffer	25 mL	-20°C	-20°C
Probe	0.4 mL	-20°C	-20°C
Converter Enzyme (Lyophilized)	1 vial	-20°C	-20°C
Developer Enzyme (Lyophilized)	1 vial	-20°C	-20°C
Substrate Mix (Lyophilized)	1 vial	-20°C	-20°C
Phosphate Standard (100 mM)	50 µL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Multi-well spectrophotometer capable of measuring fluorescence at Ex/Em= 535/587 nm
- White 96-well plate with flat bottom

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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- 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 all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
- Phosphate contamination in samples and buffers must be carefully avoided. Laboratory detergents can contain high concentrations of phosphates, so glassware must be thoroughly rinsed with distilled water to remove any phosphate bound to the glass or use disposable plastic ware.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Phosphate Assay Buffer:**
Ready to use as supplied. Store at -20 °C. Bring to room temperature before use.

9.2 **Probe:**
Ready to use as supplied. Store at -20°C. Bring to room temperature before use.

9.3 **Converter Enzyme Mix:**
Reconstitute with 220 µL Phosphate Assay Buffer. Store at -20°C; thaw before use. Use within 2 months.

9.4 **Developer Enzyme Mix:**
Reconstitute with 220 µL Phosphate Assay Buffer. Store at -20°C; thaw before use. Use within 2 months.

9.5 **Substrate Mix:**
Reconstitute with 220 µL Phosphate Assay Buffer. Store at -20°C; thaw before use. Use within 2 months.

9.6 **Phosphate Standard:**
Thaw and bring to room temperature before use. Store at -20°C.

10. Sample Preparation

10.1 Serum:

10.1.1 Serum (0.5-10 µL/well) can be directly diluted in the Assay Buffer.

10.2 Tissue and cell samples:

10.2.1 Tissues (10 - 50 mg) and cells (1 x 10^6) can be homogenized in 3 - 4 volumes of Phosphate Assay Buffer.

10.2.2 Briefly pellet at 10,000 x *g* for 10 minutes and collect the supernatant.

10.2.3 Use 1- 50 µL of supernatant and adjust to a final volume of 50 µL with Phosphate Assay Buffer

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

Δ Note: Phosphate contamination in samples and buffers must be carefully avoided. Laboratory detergents can contain high concentrations of phosphates, so glassware must be thoroughly rinsed with distilled water to remove any phosphate bound to the glass or use disposable plastic ware.

11. Standard Curve

11.1 Dilute the Phosphate Standard to 50 µM by adding 10 µL of the Phosphate Standard to 990 µl of Assay Buffer, mix well, and then add 10 µL into 190 µL of Assay Buffer, mix well.

11.2 Add 0, 2, 4, 6, 8, and 10 µL of 50 µM Phosphate Standard into a series of wells in a 96-well plate to generate 0, 0.1, 0.2, 0.3, 0.4, and 0.5 nmol/well of Phosphate Standard.

11.3 Adjust the volume to 50 µL/well with Phosphate Assay Buffer.

Standard #	Phosphate Standard (µL)	Phosphate Assay Buffer (µL)	Phosphate Standard (nmol/well)
1	10	40	0.5
2	8	42	0.4
3	6	44	0.3
4	4	46	0.2
5	2	48	0.1
6	0	50	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

Δ Note: Phosphate contamination in samples and buffers must be carefully avoided. Laboratory detergents can contain high concentrations of phosphates, so glassware must be thoroughly rinsed with distilled water to remove any phosphate bound to the glass or use disposable plastic ware.

Δ Note: White plates enhance the sensitivity of fluorescent assays and are highly recommended.

12.1.1 For Sample (S), add 1-50 µL sample per well in a white 96 well plate and adjust the volume to 50 µL with Phosphate Assay Buffer.

12.1.2 **Reaction Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Reaction Mix.

Component	Reaction Mix	Sample Background Control Mix
Phosphate Assay Buffer	40 µL	42 µL
Probe	4 µL	4 µL
Substrate Mix	2 µL	2 µL
Converter Enzyme	2 µL	--
Developer Enzyme	2 µL	2 µL

Δ Note: Xanthine and hypoxanthine in the sample will interfere with P_i in the reaction. If significant amount of them are in your sample, you may do a xanthine/hypoxanthine control by omitting the Converter Enzyme in the reaction, which will read xanthine and hypoxanthine background only. The xanthine and hypoxanthine background should be subtracted from P_i readings.

12.1.3 Add 50 µL of the Reaction Mix to each well containing the Phosphate Standard and test samples. Add 50 µL

Background Control Mix to each well containing the test samples, mix well.

12.1.4 Incubate the reaction for 30 minutes at 37°C, protected from light.

12.1.5 Measure fluorescence (Ex/Em = 535/587 nm).

13. Calculations

13.1 Subtract the 0 Phosphate Standard reading from readings.
13.2 Plot the Phosphate Standard Curve.
13.3 If sample background control is significant, then subtract sample background control reading from sample reading to obtain corrected absorbance.
13.4 Apply corrected absorbance to Standard Curve.

$$Pi\ Concentration = \frac{A}{V} = nmol/\ \mu L = \ mM$$

A = P_i amount in the reaction from standard curve (in nmol),
V = sample volume added into the reaction well (in µL).
D = Sample dilution factor

Phosphate standard (NaH_2PO_4) molecular weight: 119.98 g/mol

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

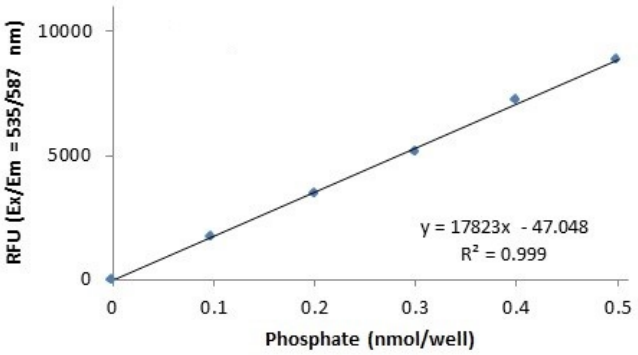

Figure 1. Phosphate standard curve.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. 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)