Product Information

Phosphate Assay Kit (Fluorometric)

I. Kit Contents:

Components	K2076-100 100 assays	Cap Color	Part Number
Phosphate Assay Buffer	25 ml	WM	K2076-C-1
PicoProbe™ (in DMSO)	200 µl	Blue	K2076-C-2
Converter	1 vial	Brown	K2076-C-3
Developer	1 vial	Green	K2076-C-4
Phosphate Substrate	220 µl	Purple	K2076-C-5
Phosphate Standard (100 mM)	50 µl	Yellow	K2076-C-6

II. Introduction:

Phosphate is an important inorganic ion in biological systems and has many functions. Phosphate can turn on or off enzyme activity through the mediation of the various phosphatases and protein kinases in biological systems. Phosphate also plays an important role in mineralization processes and is a major stimulus of algal blooms mainly found in bodies of fresh water, because of run-off from areas of high fertilizer use.

The Phosphate Assay Kit (Fluorometric) provides a highly sensitive, easy and fast way for detection of phosphate (Pi) over a wide range of concentrations in various samples based on fluorometric method. In the presence of a proprietary enzyme, inorganic phosphate reacts with sucrose to produce glucose-1-phosphate, which is specifically oxidized to yield a product that reacts with PicoProbe™ to produce fluorescence (Ex/Em = 535/587 nm). The kit can be used to detect phosphate produced through reactions involving GTPases, ATPases, acid and alkaline phosphatases, phosphorylase, 5'-nucleotidase, protein phosphatases, etc. from a variety of samples. In addition, the assay is not affected by the presence of glucose in samples. This kit can detect phosphate concentrations between 2 µM and 10 µM, with a lower limit of approximately 100 pmol.

III. Storage and Handling:

Store kit at -20℃, protected from light. Allow the reagents to warm to room temperature and briefly centrifuge the vials prior to opening. Read the entire protocol before the assay. A 96-well white plate is recommended for this assay.

IV. Reagent Preparation:

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

Converter, Developer: Resuspend in 220 µl Assay Buffer, respectively. Aliquot and store at -70℃. Avoid repeated freeze thaw. Use within two months.

V. Phosphate Assay Protocol:

Note: Phosphate contamination in samples must be carefully avoided. Laboratory detergents can contain large amount of phosphate, therefore glassware must be thoroughly rinsed with distilled water before use.

1. Standard Curve Preparations:

Dilute the Phosphate Standard to 100 µM by adding 10 µl of the Phosphate Standard 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 and 10 µl of 100 µM standard into each well individually. Adjust the volume to 50 µl/well with Assay Buffer to generate 0, 200, 400, 600, 800, 1000 pmol/well of the Phosphate Standard.

2. Sample Preparation: Add 1 – 50 µl test samples in a 96-well plate; if using serum sample, serum (0.5 - 2 µl/well) can be directly diluted in the Assay Buffer. Bring the volume to a total of 50 µl/well with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the linear range of the standard curve.

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

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

Note: You may do a control (optional) by omitting the Converter in the reaction, which will read the no-converter background. If the reading is higher than the 0 phosphate control, then the background should also be subtracted from Pi readings.

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

5. Incubate the reaction for 1 hour at room temperature, protected from light.

6. Measure the fluorescence at Ex/Em = 535/587 nm in a micro plate reader.

7. Correct the background by subtracting the value derived from the 0 phosphate control from all readings. Plot the Pi Standard Curve. Apply the sample readings to the standard curve.

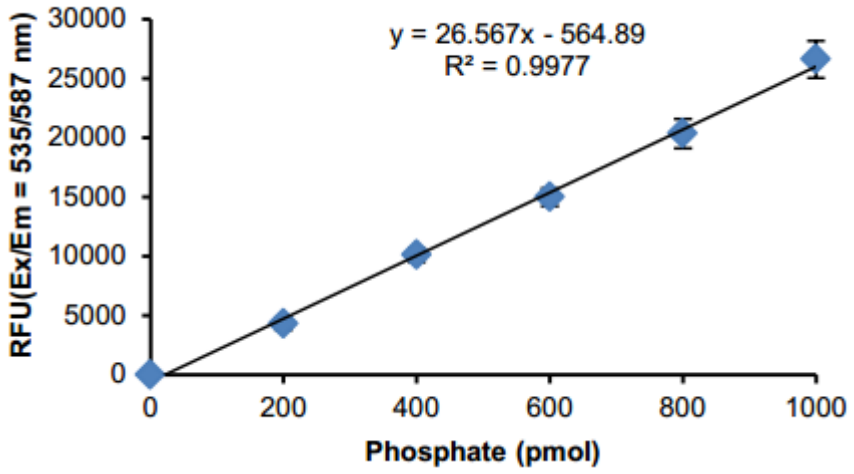

Figure: Phosphate standard curve made according to the protocol (n = 3, error bars represent standard deviation).

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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