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PicoProbe™ Phosphate Fluorometric Assay Kit

(Catalog #K419-100; 100 Assays; Store kit at -20˚C)

I. Introduction:

Inorganic phosphate (P_i) 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. The P_i ion is also of great importance in mineralization processes and is a primary stimulus of algal blooms frequently found in bodies of fresh water, due to runoff from areas of high fertilizer use. The PicoProbe Phosphate Assay Kit 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 PicoProbe™ 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 PicoProbe assay is not affected by the presence of glucose in samples (Note: Glucose interferes with many other commercially available assays). This PicoProbe assay is highly sensitive with the detection limit of approximately 40 pmol/well.

II. Kit Contents:

Components	100 assays	Cap Code	Part Number
Phosphate Assay Buffer	25 ml	WM	K419-100-1
PicoProbe™	0.4 ml	Blue	K419-100-2
Converter Enzyme	1 vial	Green	K419-100-3
Developer Enzyme	1 vial	Brown	K419-100-4
Substrate Mix	1 vial	Purple	K419-100-5
Phosphate Standard (100 mM)	50 µl	Yellow	K419-100-6

III. Storage and Handling:

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

IV. Reagent Preparation:

Converter Enzyme, Developer Enzyme, and Substrate Mix: Dissolve in 220 µl Assay Buffer separately. Aliquot and store at -20°C. Use within two months.

V. Assay Protocol:

***Caution**: 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.

1. Standard Curve Preparations:

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. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of the Phosphate Standard.

2. Sample Preparations:

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 samples, serum* (0.5-10 µl/well) can be directly diluted in the Assay Buffer. Tissues (10 - 50 mg) and cells (1 x 10^6) can be homogenized in 3 - 4 volumes of Phosphate Assay Buffer. Briefly pellet at 10,000 x g for 10 min and collect supernatant. Use 1-50 µl of supernatant and adjust to a final volume of 50 µl with Phosphate Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.

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

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:

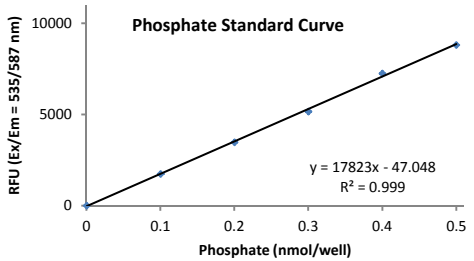
Reaction Mix		Sample Background Control	
40 µl	Assay Buffer	42 µl	Assay Buffer
4 µl	PicoProbe™	4 µl	PicoProbe™
2 µl	Substrate Mix	2 µl	Substrate Mix
2 µl	Converter Enzyme	----------	----------
2 µl	Developer Enzyme	2 µl	Developer Enzyme

***Note:** Xanthine and hypoxanthine in the sample will interfere with Pi 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.

4. Add 50 µl of the Reaction Mix to each well containing the Phosphate Standard and test samples, and add 50 µl Background Control to each well containing the test samples, mix well. Incubate the reaction for 30 min at 37°C, protected from light.
5. Measure fluorescence at Ex/Em = 535/587 nm in a micro plate reader.
6. **Calculations:** Correct background by subtracting the value derived from the background control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot the P_i Standard Curve and apply the sample readings to the standard curve.

P_i Concentration = A/V nmol/µl or mM

Where: **A** is the P_i amount in the reaction from standard curve (in nmol),
V is sample volume added into the reaction well (in µl).
Phosphate standard (NaH_2PO_4) molecular weight = 119.98 g/mol



RELATED PRODUCTS:

Alkaline Phosphatase Assay Kit	Acid Phosphatase Assay Kit
ADP/ATP Ratio Assay Kit	Phosphate Colorimetric Assay Kit
NAD/NADH Quantification Kit	NADP/NADPH Quantification Kit
Pyruvate Assay Kit	Lactate Assay Kits
Ammonia Assay Kit	Glutamate Assay Kit
Glucose Assay Kit	Fatty Acid Assay Kit
Ethanol Assay Kit	Uric Acid Assay Kit
Glycogen Assay Kit	Sucrose Assay Kit
Sarcosin Assay Kit	Cholesterol Assay Kit
Creatine & Creatinine Assay Kits	HDL/LDL Assay Kits

FOR RESEARCH USE ONLY! Not to be used on humans.

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates or white plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		