For research use only

Fluorescent Phosphate Assay Kit

(Catalog #K420-100; 100 Assays; Store kit at -20°C)

I. Introduction:

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. The newly designed Phosphate Assay Kit provides a sensitive, easy, quick means of assessing phosphate over a wide range of concentrations. In the assay, inorganic phosphate reacted with maltose to produce glucose in the present of a special enzyme. The glucose is specifically oxidized to generate a product that reacts with the OxiRed® 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. Glucose background can be eliminated by doing a glucose control. Phosphate concentrations between 0.4 µM and 10 µM, with a lower detection limit of approximately 40 pmol can be directly determined.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Phosphate Assay Buffer	25 ml	WM	K420-100-1
OxiRed® Probe (in DMSO)	200 µl	Red	K420-100-2A
Maltose Converter	1 vial	Blue	K420-100-4
Glucose Developer	1 vial	Green	K420-100-5
Phosphate Substrate	0.2 ml	Purple	K420-100-6
Phosphate Standard (100 mM)	50 µl	Yellow	K420-100-7

III. Storage and Handling:

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.

IV. Reagent Preparation:

OxiRed® Probe: Dissolve in 220 µl DMSO (provided) before use. Store at –20°C, protect from light and moisture. Use within two months.

Maltose Converter, Glucose Developer: Dissolve in 220 µl Assay Buffer separately. Aliquot and store at –20°C. Use within two months.

V. Phosphate 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. Standard Curve Preparations:

Dilute the Phosphate Standard to 25 µM by adding 10 µl of the Phosphate Standard to 990 µl of Assay Buffer, mix well, then take 10 µl into 390 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, 50, 100, 150, 200, 250 pmol/well of the Phosphate Standard.

2. Sample Preparation: Add 1 – 50 µl test samples in a 96-well plate; bring the volume to a total of 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.

*Serum contains 4-6 mM glucose which interferes with the result, please perform a glucose control.

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:

45 µl	Assay Buffer
0.2 µl	OxiRed® Probe
1 µl	Phosphate Substrate
2 µl	Maltose Converter*
2 µl	Glucose Developer

***Note:** Glucose increases the background in this reaction. If a significant amount of glucose is in your sample, you may do a glucose control by omitting the Maltose 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.

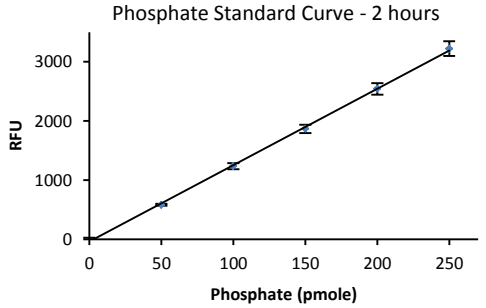
5. Incubate the reaction for 2 hours at 37°C, protect from light.

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

7. Correct background by subtracting the value derived from the 0 phosphate control from all sample readings (Note: 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.

Pi 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).



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

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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 ; 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.		