

# 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<sup>®</sup> 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 <sup>®</sup> 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<sup>®</sup> 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:

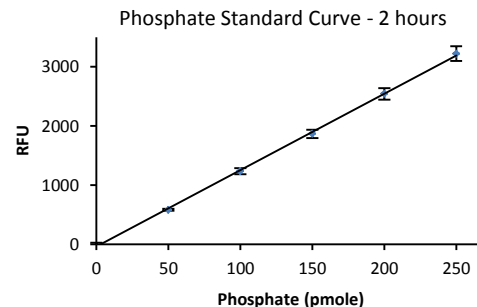
- 45 μl Assay Buffer
- 0.2 μl OxiRed<sup>®</sup> Probe
- 1 μl Phosphate Substrate
- 2 μl Maltose Converter\*
- 2 μl Glucose Developer

\*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:**

- |   |  |
|---|--|
| <ul style="list-style-type: none"> <li>Alkaline Phosphatase Assay Kit</li> <li>ADP/ATP Ratio Assay Kit</li> <li>NAD/NADH Quantification Kit</li> <li>Pyruvate Assay Kit</li> <li>Ammonia Assay Kit</li> <li>Glucose Assay Kit</li> <li>Ethanol Assay Kit</li> <li>Glycogen Assay Kit</li> </ul> | <ul style="list-style-type: none"> <li>Acid Phosphatase Assay Kit</li> <li>Phosphate Colorimetric Assay Kit</li> <li>NADP/NADPH Quantification Kit</li> <li>Lactate Assay Kits</li> <li>Glutamate Assay Kit</li> <li>Fatty Acid Assay Kit</li> <li>Uric Acid Assay Kit</li> <li>Sucrose Assay Kit</li> </ul> |
|---|--|

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

## GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Samples were not deproteinized (if indicated in datasheet)</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed, deproteinize samples</li> <li>• Use fresh samples or store at correct temperatures till use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>
<p><b>Note:</b> The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		