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Product Information

Phosphate Fluorometric Assay Kit

Catalog Number **MAK031**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Phosphate is an essential component in living organisms and contributes to a variety of biological functions, including structural roles within nucleic acids, cellular membranes, and bone. Phosphate is also important in the transport of cellular energy, nucleic acid metabolism and signal transduction. Hyperphosphatemia, a condition of excess phosphate levels in the blood, can lead to calcification of organs and interference with usage of other inorganic ions, such as iron, calcium, magnesium, and zinc.

The Phosphate Fluorometric Assay Kit provides a simple and direct procedure for measuring phosphate in a variety of samples. Phosphate reacts with sucrose to produce glucose-1-phosphate in the presence of an enzyme. The glucose-1-phosphate is subsequently oxidized and reacts with a probe, resulting in a fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product proportional to the amount of phosphate present. Unlike other commercially available assays, the assay is not affected by the presence of glucose in samples. This kit has a linear range of detection between 50–250 pmoles.

Components
The kit is sufficient for 100 assays in 96 well plates.

Phosphate Assay Buffer 25 mL
Catalog Number MAK031A

Fluorescent Peroxidase Substrate 0.2 mL
Catalog Number MAK031B

Converter 1 vl
Catalog Number MAK031C

Developer 1 vl
Catalog Number MAK031D

Phosphate Substrate 0.2 mL
Catalog Number MAK031E

Phosphate Standard, 100 mM 50 µL
Catalog Number MAK031F

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening.
Note: Many laboratory detergents contain high levels of phosphates, which can adhere to cleaned glassware. It is highly recommended to use disposable plastic labware for all samples, standards, and reagents to avoid contamination.

Assay Buffer, Fluorescent Peroxidase Substrate, Phosphate Substrate, Phosphate Standard – Allow to warm to room temperature before use.

Converter and Developer – Reconstitute each in 220 µL of Assay Buffer. Aliquot and store at –20 °C. Stable for 2 months when stored at –20 °C.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Phosphate Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM Standard with 990 µL of Phosphate Assay Buffer. Further dilute 20 µL of diluted standard with 180 µL of assay buffer to prepare a 100 µM solution. Add 0, 2, 4, 6, 8, and 10 µL of the 100 µM standard solution into a 96 well plate, generating 0 (blank), 200, 400, 600, 800, and 1000 pmole/well standards. Add water to each well to bring the volume to 50 µL.

Sample Preparation
Add up to 50 µL of sample to wells. If using serum sample, add 0.5–2 µL of serum to wells. Bring samples to a final volume of 50 µL with Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	**Samples and Standards**	**Sample Blank**
Phosphate Assay Buffer	43 µL	45 µL
Fluorescent Peroxidase Substrate	1 µL	1 µL
Phosphate Substrate	2 µL	2 µL
Converter	2 µL	–
Developer	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for1 hour at room temperature. Protect the plate from light during the incubation.

3. Measure the fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) Phosphate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate Phosphate standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Subtract the sample blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of phosphate present in the samples may be determined from the standard curve.

Concentration of Phosphate

$$S_a/S_v = C \text{ (nmole/}\mu\text{L, or mM)}$$

S_a = Amount of phosphate in unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added to reaction well
C = Concentration of phosphate in sample

Sample Calculation

Amount of phosphate (S_a) = 2.84 nmole (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of phosphate in sample
2.84 nmole/50 μL = 0.0568 nmole/μL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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