Phosphorus Assay Kit

Catalog No.: abx298874

Size: 100 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of Phosphorus concentrations in serum, saliva, urine, and other biological fluids.

Detection Range: 0.01 mmol/L – 0.4 mmol/L

Introduction: Phosphorus, in the form of inorganic phosphate ions, is an essential element in all living organisms. Adenosine triphosphate (ATP) is widely used as an energy source in many biological processes and has a role in phosphorylating proteins, triggering signaling cascades. Phosphorus is also found in bones and teeth. Various diseases, such as liver disease and vitamin D deficiency, can result in insufficient phosphorus (hypophosphatemia) or elevated phosphorus levels (hyperphosphatemia).

Abbexa's Phosphorus Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Phosphorus concentrations. Phosphate ions react with ammonium molybdate to form a blue-colored product with an absorption maximum at 620 nm. The intensity of the color is proportional to the concentration of phosphorus, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 5 ml
4. Dye Reagent: 1 vial
5. Standard (0.4 mmol/L): 1 ml

Materials Required But Not Provided

1. Microplate reader (620 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Solution**
  Add 5 ml of distilled water into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

2. Sample

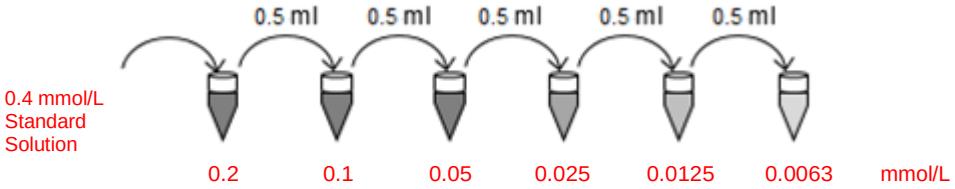
- **Liquid samples**
  In a microcentrifuge tube, add 100 µl of sample and 900 µl of Assay Buffer, and mix thoroughly. Centrifuge at 8000 × g at 25°C for 10 minutes. Transfer the supernatant to a new tube, then analyze immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 6 tubes with 0.2 mmol/L, 0.1 mmol/L, 0.05 mmol/L, 0.025 mmol/L, 0.0125 mmol/L, and 0.0063 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 0.4 mmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 50 µl of Reaction Buffer to all wells.
4. Add 50 µl of Dye Reagent Solution to all wells.
5. Add 100 µl of distilled water to the blank wells.
6. Add 100 µl of prepared standards to the standard wells.
7. Add 100 µl of prepared samples to the sample wells.
8. Tap the plate gently to mix. Allow to stand for 10 minutes.
9. Read and record absorbance at 620 nm.

C. Calculations

Phosphorus concentration per ml sample:

$$\textbf{Phosphorus}\ (\textbf{mmol/ml}) = 10 \times C_{Standard} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = 4 \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Standard}$ Concentration of highest standard (0.4 mmol/L)