Phosphorus (Pi) Assay Kit

Catalog No.: abx294160

Size: 100 tests

Detection Range: 0.005 mmol/L - 2.0 mmol/L

Sensitivity: 0.005 mmol/L

Storage: Store all components at 2-8°C for 12 months. Store the Chromogenic Reagents B and C in the dark.

Application: For detection and quantification of Phosphorus (Pi) concentration in serum, plasma, tissue homogenates, and other biological fluids.

Introduction

Inorganic phosphorus (Pi) is an anion that is used in many key biological processes such as ATP formation and skeletal growth. Abbexa's Phosphorus (Pi) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating phosphorus (Pi) concentrations. Inorganic phosphorus can react with molybdic acid to produce phosphomolybdic acid. With the addition of a reducing agent, the phosphomolybdic acid can be reduced to molybdenum blue, which has a maximum absorption peak of 660 nm. By measuring the OD value at 660 nm, the phosphorus concentration can be indirectly calculated.

Kit components

1. Chromogenic Reagent A: 50 ml
2. Chromogenic Reagent B: 4 vials
3. Chromogenic Reagent C: 2 vials
4. Protein Precipitator: 60 ml
5. Phosphorus Standard (10 mmol/L): 1 ml

Materials Required But Not Provided

1. Spectrophotometer (660 nm)
2. Double Distilled Water
3. Normal saline (0.9% NaCl)
4. Water bath
5. Centrifuge
6. Micropipettor
7. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate (initial recommendation 20 mg). For each 1 g of homogenate, add 9 ml of normal saline. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with double distilled water, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Human plasma	1
10% Mouse kidney tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Mouse liver tissue homogenization	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Chromogenic Reagent B:** Add 12.5 ml of double distilled water to one vial of Chromogenic Reagent B. Mix thoroughly. Store at 2-8°C for up to 5 days.

- **Chromogenic Reagent C:** Dilute one vial of Chromogenic Reagent C with 25 ml of double distilled water. Mix thoroughly. Store at 2-8°C for up to 2 months.
- **Chromogenic Reagent Working Solution:** For each well, prepare Chromogenic Reagent Working Solution by mixing double distilled water, Chromogenic Reagent A, Chromogenic Reagent B working solution, Chromogenic reagent C working solution in a 2:1:1:1 ratio, respectively. For example, to make 2 ml of Chromogenic Reagent Working solution, mix 0.8 ml double distilled water, 0.4 ml of Chromogenic Reagent A, 0.4 ml of Chromogenic Reagent B working solution and 0.4 ml of Chromogenic Reagent C working solution**.** The Chromogenic Reagent should be prepared immediately prior to starting the assay.
- **0.5 mmol/L standard solution**: For each well, prepare 200 µl of 0.5 mmol/L standard solution (mix well 10 µl of 10 mmol/L phosphorus standard and 190 µl of double distilled water). The 0.5 mmol/L standard solution should be prepared immediately prior to starting the assay.

B. Assay Procedure

1. **Sample Supernatant:** Take 0.1 ml of sample, then add 0.4 ml of protein precipitator, mix fully. Centrifuge at 1100 × g for 10 mins and take the supernatant for detection.
2. **Sample Measurement:**

   2.1 Add 0.2 ml of 0.5 mmol/L standard solution to the standard tubes.

   2.2 Add 0.2 ml of sample supernatant to the sample tube.

   2.3 Add 0.2 ml of double distilled water to the blank EP tube.

   2.4 Add 2.0 ml of Chromogenic Reagent to each tube and mix well.

   2.5 Incubate the tubes at 37°C for 30 min, then cool the tubes to room temperature.

   2.6 Zero the spectrophotometer with double distilled water, and then measure the OD at 660 nm with 1 cm optical path quartz cuvette.

C. Calculation of Results

1. Serum and plasma samples:

$$\text{Pi (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times C_{\text{Standard}} \times 5 \times f$$

2. Tissue samples:

$$\text{Pi (mmol/g protein)} = \frac{\Delta A_1}{\Delta A_2} \times \frac{C_{\text{Standard}}}{C_{\text{Protein}}} \times 5 \times f$$

where:

ΔA_1	$OD_{Sample} - OD_{Blank}$
ΔA_2	$OD_{Standard} - OD_{Blank}$
$C_{Standard}$	Concentration of standard (0.5 mmol/L)
5:	Dilution factor of sample before carrying out the assay
$C_{Protein}$	Concentration of protein in sample (g protein/L)

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.