MANUAL

Phosphorus (Inorganic) Colorimetric Assay Kit

Catalog #: MA-PHOS-2

ISO 13485:2016

Introduction

Phosphorus 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. Phosphorus is also important in cellular energy transport, nucleic acid metabolism, and signal transduction. Hyperphosphatemia, a condition of excessive levels of phosphorus in the blood, can lead to organ calcification and interfere with the utilization of other inorganic ions, such as iron, calcium, magnesium, and zinc.

RayBio® Phosphorus (Inorganic) Colorimetric Assay Kit provides a simple, reproducible, and sensitive tool for measuring inorganic phosphorus concentration in serum, cell lysates, urine, saliva, and other biological liquid samples. In this assay, inorganic phosphorus reacts with ammonium molybdate in an acid medium to form a phosphomolybdate complex that absorbs light at 340nm. The absorbance at this wavelength is directly proportional to the amount of inorganic phosphorus present in the sample.

Storage

The entire kit may be stored at 2–8 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate	Two 96-well (12 strips x 8 wells) plates	RT*
Phosphorus Standard	1 vial (500 µl of 650 µM)	2–8 °C
Assay Buffer	2 × 16 ml	2–8 °C

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 340 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, Phosphorus levels in human adult serum are in the range of 2.5-4.8 mg/dL (0.81-1.55 mM). Serum samples can be diluted at least 1:10 with deionized water.

3. Urine samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. Urine samples can be diluted at least 1:100 with deionized water.

4. Saliva samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. Saliva samples can be diluted at least 1:50 with deionized water.

NOTE:

1. *Plasma should not be used since anticoagulants may produce falsely low values.*
2. *Optimal experimental conditions for samples must be determined by the investigator. A set of serial dilutions is recommended for samples to achieve optimal assay results.*

Standard Preparation

To prepare a dilution series of standard in the concentration range of 0 µM – 400 µM (see Table below),

1. Label 8 microtubes #1 through 8 which with the following concentrations: 400, 200, 100, 50, 25, 12.5, 6.25, 0 µM.
2. Pipette 153.8 µL deionized water into labeled tube #1, and 200 µL deionized water into labeled tubes #2 – tube #8.
3. Pipette 246.2 µL of the 650 µM Phosphorus Standard (provided) into tube #1, Mix thoroughly to make the 400 µM Phosphorus Standard.
4. To make the 200 µM standard, pipette 200µL tube #1 into the tube labeled #2. Mix thoroughly.
5. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 200 µl of the prior concentration until the 6.25 µM is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh, mixed thoroughly, and used immediately.

Labeled Tubes	Phosphorus Standard (µL)	Deionized Water (µL)	Standard Conc. (µM)
1	246.2 µL of 650 µM Stock	153.8 µL	400
2	200 µL of Tube #1	200 µL	200
3	200 µL of Tube #2	200 µL	100
4	200 µL of Tube #3	200 µL	50
5	200 µL of Tube #4	200 µL	25
6	200 µL of Tube #5	200 µL	12.5
7	200 µL of Tube #6	200 µL	6.25
8	0µL	200 µL	0

Assay Procedure

Each phosphorus standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 50 µL of the diluted phosphorus standards or samples to the 96-well Microplate.
2. Initiate the reaction by adding 150 µL Assay Buffer to each well.
3. Cover with the plate cover. Carefully shake the plate for a few seconds to mix.
4. Incubate the plate for 30 minutes at room temperature in the dark.
5. Measure the absorbance at 340nm using a plate reader.

Calculation of Results

Subtract the blanks

Average the absorbance value of the blank wells (Standard 0 µM) and subtract this from the absorbance values of all the other wells. These are the corrected absorbance.

Plotting the standard curves

Make a plot of corrected absorbance at 340nm as a function of phosphorus concentration.

Determination of sample Phosphorus concentration

Phosphorus (µM) = $\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$

OD_{Sample} = Optical density (OD) reading of the Sample

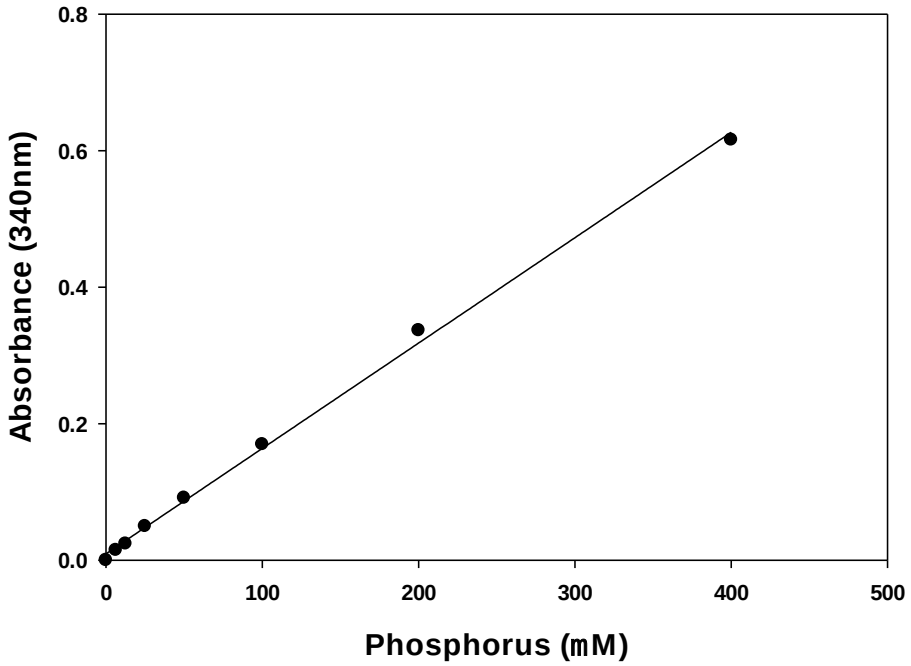
OD_{Blank} = Optical density (OD) reading of the Blank (Standard 0 µM)

Slope is from the plot of Phosphorus concentration vs. Absorbance shown in Typical data below

DF = Sample Dilution factor (DF = 1 for undiluted samples)

Note: If the calculated phosphorus concentration of the sample is higher than 400 µM, dilute the sample in Deionized water and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Sensitivity

The minimum detectable concentration of phosphorus is 3.1 µM.
Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank.

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 1.4%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 3.3%.

This product is for research use only.