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User's Manual and Instructions

Phosphate Assay Kit (Z5030017)

Quantitative Colorimetric Phosphate Determination at 620nm

DESCRIPTION

Phosphate (Pi) is one of the most important ion species in nature. Phosphate is present in all biological systems. It is a major constituent in minerals and fertilizers, and is a component of industrial wastewater. Thus accurate determination of phosphate concentration finds numerous applications in pharmacology, biomedical research, clinical chemistry, industrial process monitoring and environmental monitoring.

Simple, direct and automation-ready procedures for measuring phosphate concentration in biological and environmental samples are becoming popular. Biochain's phosphate assay kit is designed to measure phosphate ion directly in samples without any pretreatment. The improved Malachite Green method utilizes the malachite green dye and molybdate, which forms a stable colored complex specifically with inorganic phosphate. The intensity of the color, measured at 620nm, is directly proportional to the phosphate concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

KEY FEATURES

Sensitive and accurate. Linear detection range 0.30 μM (0.0028 mg/dL) to 50 μM (0.47 mg/dL) phosphate in 96-well plate assay.
Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 30 min. Can be readily automated as a high-throughput assay for thousands of samples per day.
Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Assays can be executed in cuvet or 96-well plate.
Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on raw biological samples i.e., in the presence of lipid, protein and minerals.

APPLICATIONS

Direct Assays: Pi in serum, urine, saliva, sweat, tissue culture etc.
Drug Discovery/Pharmacology: effects of drugs on Pi metabolism.
Food and Beverages: Pi determination.
Environment: Pi determination in water, soil and fertilizer.

KIT CONTENTS (500 tests in 96-well plates)

Reagent: 50 mL
Pi standard: 14 mL 0.28 mg/dL (30 μM)
Blank Control: 14 mL

Storage conditions. Store Reagent, standard and blank control at 4°C. Shelf life: 12 months.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation:

Important: bring reagents to room temperature and shake well before use.

Procedure using 96-well plate:

1. Set up standards and samples. Transfer 50 μL distilled water ("Blank"), Standard and samples in duplicate wells of a clear bottom 96-well plate.
2. Add 100 μL Reagent and tap lightly to mix.
3. Incubate 30 min at room temperature and read optical density at 620nm (600-660nm). Signal is stable for at least 60 min.

Procedure using cuvette:

1. Set up test tubes labeled Blank, Standard, Samples. Transfer 400 μL Water, Standard and samples to appropriately labeled tubes.
2. Add 800 μL Reagent and tap lightly to mix.
3. Incubate 30 min at room temperature, transfer to cuvet and read optical density at 620 nm (600-660nm). Signal is stable for > 60 min.

Important: (1) if sample OD is higher than the OD for standard, dilute samples in distilled water and repeat the assay. (2) It is not necessary to prepare a calibration curve, because the concentration of the provided standard lies within the linear range. (3) Precipitation may occur at high concentrations of phosphate (>100 μM), or in the presence of high concentrations of e.g. proteins and metals. In this case, dilute samples in distilled water and repeat the assay.

CALCULATION

The phosphate concentration of Sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{BLANK}} \times 0.28 \text{ (mg/dL)}$$

OD_{BLANK}, $OD_{STANDARD}$ and OD_{SAMPLE} are OD_{620nm} values of Blank, Standard and Sample, respectively.
Conversions: 1 mg/dL Pi equals 106.4 μM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories.

Procedure using 96-well plate:

Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

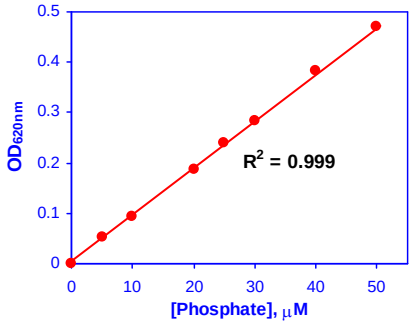
Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD 620nm.

EXAMPLES (96-well plate assay):

	Pi (mg/dL)
1	6.4 ± 0.6
2	2.2 ± 0.1
3	3.5 ± 0.1
4	0.081 ± 0.003
5	0.003 ± 0.001
6	0.02 ± 0.001
7	1.10 ± 0.01
8	0.56 ± 0.06
9	0.19 ± 0.03

Biological Samples: 1. Commercial 2% reduced fat milk (Kirkland). 2. Invitrogen fetal bovine serum. 3. Fresh human urine. **Water samples:** 4. Tap water (Hayward, CA). 5. Tap water (San Bruno, CA). **Food and Beverages:** 6. Crystal Geyser natural alpine spring water. 7. Coca-cola® classic coke. 8. Lipton Lemon iced tea. **Environmental:** 9. Soil extract. 5.6 g of soil (Hayward, CA) was extracted with 10 mL MilliQ water. The supernatant was centrifuged to remove any insoluble particles. Clear supernatant was assayed.



Calibration curve in 96-well plate

LITERATURE

1. Cogan EB et al (1999). A robotics-based automated assay for inorganic and organic phosphates. *Anal Biochem.* 271(1):29-35.

2. Ekman P, Jager O (1993). Quantification of subnanomolar amounts of phosphate bound to seryl and threonyl residues in phosphoproteins using alkaline hydrolysis and malachite green. *Anal Biochem.* 214(1):138-141.

3. Fisher DK, Higgins TJ (1994). A sensitive, high-volume, colorimetric assay for protein phosphatases. *Pharm Res.* 11:759-763.