

Phosphate Assay Kit (Colorimetric)

LS-K287-500 (500 Tests) • Store at 4°C



Introduction

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. This 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 0.3 μ M (0.0028 mg/dL) to 50 μ M (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 cuvette 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.

Components

Component	K287-500
	500 Tests
Reagent	50 mL
Blank Control	14 mL
Pi standard (0.28 mg/dL (30 μ M))	14 mL

Materials Not Supplied

Pipetting 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 cuvettes for measuring OD 620nm.

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121

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Storage

The kit is shipped at room temperature. Store all components at 4°C. Shelf life: 12 months after receipt.

Assay Procedure

Reagent Preparation

Important: bring reagents to room temperature and shake 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).

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 cuvette and read optical density at 620 nm (600-660nm).

Important: (1) if sample phosphate concentration is higher than 50 µM, 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.

Calculations

The phosphate concentration of Sample is calculated as

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

OD_{BLANK}, OD_{STANDARD} and OD_{SAMPLE} are OD_{620nm} values of Blank, Standard and Sample, respectively.

Conversions: 1 mg/dL Pi equals 105.3 µM, 0.001% or 10 ppm.

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Sample Data

96-well plate assay

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

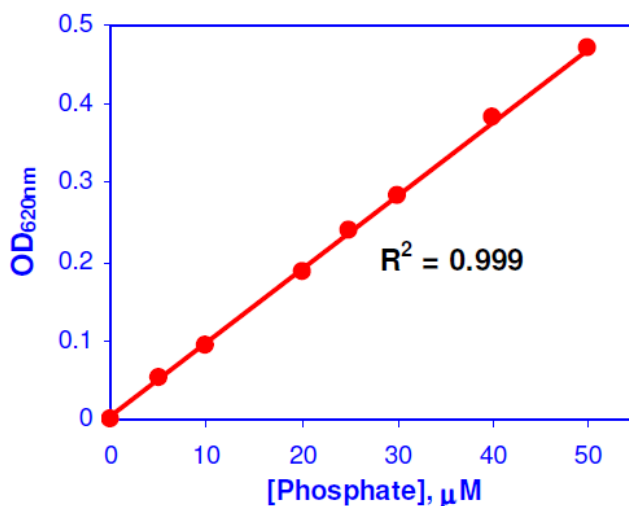
water. The supernatant was

centrifuged to remove any

insoluble particles. Clear

supernatant was assayed.

	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



Standard Curve in 96-well plate assay

Version: V.08.09.2018

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