Technical Bulletin

Phosphate Assay Kit

Catalogue Number MAK488

Product 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. 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 useful in research studies. The Phosphate Assay Kit is designed to measure phosphate ions directly in samples without any pretreatment. The improved Malachite Green method utilizes malachite green and molybdate, which forms a stable colored complex specifically with inorganic phosphate. The intensity of the color, measured at 620 nm, is directly proportional to the phosphate concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

The Linear detection range of the kit is 0.30 - 50 µM (0.0028 - 0.47 mg/dL) phosphate. The kit is suitable for the quantification of phosphate in serum, urine, saliva, sweat, food and beverages, water, soil, and fertilizer, as well as studying the effects of drugs on phosphate metabolism.

Components

The kit is sufficient for 500 colorimetric assays in 96-well plates.

- Reagent 50 mL

  Catalogue Number MAK488A
- Blank Control 14 mL

  Catalogue Number MAK488B
- Pi Standard (0.28 mg/dL [30 µM]) 14 mL

  Catalogue Number MAK488C

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.

For Cuvette Method Only

- Cuvettes suitable for measuring optical density at 620 nm
- Spectrophotometer
- Sample tubes

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped in room temperature. Store components at 2-8 °C.

Preparation Instructions

Equilibrate reagents to room temperature and shake prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Sample pre-treatment is not required.

Procedure Using 96-Well Plate

1. Transfer 50 µL of Blank Control into duplicate wells of a 96-well plate for use as the Blank.
2. Transfer 50 µL of Pi Standard into duplicate wells of the plate. It is not necessary to prepare a calibration curve, because the concentration of the provided Standard lies within the linear range of the reaction.
3. Transfer 50 µL of Sample into duplicate wells of the plate.
4. Add 100 µL of Reagent to each well and tap lightly to mix.

   Note: Precipitation may occur at high concentrations of phosphate (>100 µM) or in the presence of high concentrations of proteins and metals. If precipitation occurs, dilute Samples in purified water and repeat the assay. Multiply the results by the dilution factor (DF).

5. Incubate the plate for 30 minutes at room temperature.
6. Measure the optical density (OD) at 620 nm.

Procedure Using Cuvettes

1. Label tubes for Blank, Standard and Sample.
2. Transfer 400 µL of Blank Control into duplicate Sample tubes for use as the Blank.
3. Transfer 400 µL of Pi Standard into duplicate tubes. It is not necessary to prepare a calibration curve, because the concentration of the provided Standard lies within the linear range of the reaction.
4. Transfer 400 µL of Sample into duplicate tubes.
5. Add 800 µL of Reagent to each tube and mix.

   Note: Precipitation may occur at high concentrations of phosphate (>100 µM) or in the presence of high concentrations of proteins and metals. If precipitation occurs, dilute Samples in purified water and repeat the assay. Multiply the results by the dilution factor (DF).

6. Incubate tubes for 30 minutes at room temperature.
7. Transfer to cuvettes and measure the optical density (OD) at 620 nm.

Results

Calculate phosphate concentration of the Sample using the below formula.

Phosphate (mg/dL) =

$$\frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times 0.28 \times DF$$

where:

OD_{Sample} = Optical density reading of Sample

$OD_{Standard}$ = Optical density reading of Standard

OD_{Blank} = Optical density reading of Blank

0.28 = Concentration of the Standard in mg/dL

DF = Sample dilution factor (DF = 1 for undiluted Samples)

If Sample phosphate concentration is higher than 50 µM, dilute in purified water and repeat the assay. Multiply the results by the dilution factor (DF).

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

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