Phosphate Assay Kit (Colorimetric)

LS-K328-500 (500 Tests) • **Store at 4°C**

Introduction

The Phosphate Assay Kit is based on a proprietary formulation of the malachite green dye. The reagent forms a blue colored complex with free orthophosphate. The rapid color formation from the reaction can be conveniently measured on a spectrophotometer (600 - 660 nm) or on a plate reader. The non-radioactive colorimetric assay kits have been optimized to offer superior sensitivity and prolonged shelf life. The assay is simple and fast, involving a single addition step for phosphate determination. Assays can be performed in tubes, cuvettes or multi-well plates. The assays can be conveniently executed in 96-well plates for high-throughput screening of enzyme inhibitors.

Key Features

- Reagent very stable. Due to our innovative formulation, no precipitation of reagent occurs. Therefore no filtration of reagent is needed prior to assays, as is often required with other commercial kits.
- High sensitivity and wide detection range: detection of as little of 20 pmoles of phosphate and useful range between 0.4 µM and 50 µM phosphate.
- Fast and convenient: single reagent "mix-and-measure" assay allows quantitation of free phosphate within 30 minutes.
- Compatible with routine laboratory and HTS formats: assays can be performed in tubes, cuvettes or microplates, on spectrophotometers and plate readers.
- Robust and amenable to HTS: Z' factors of 0.7 to 0.9 are observed in 96-well plates. Can be readily automated on HTS liquid handling systems.

Applications

- Phosphatase Assays: liberation of phosphate from peptide, protein or small molecule substrate.
- Lipase Assays: liberation of phosphate from phospholipids.
- Nucleoside Triphosphatase Assays: liberation of phosphate from nucleoside triphosphates (ATP, GTP, TTP, CTP, etc.).
- Quantitation of Phosphate in phospholipids, proteins and DNAs, etc.
- Drug Discovery: high-throughput screen for phosphatase inhibitors.

Components

Component	K328-500
	500 Tests
Reagent	50 mL
Standard (1mM phosphate)	1 mL

Storage

The kit is shipped at ambient temperature. The Blue Reagent and standard are stable for 12 months when stored at 4°C.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

Phosphate Assay Kit (Colorimetric)

LS-K328-500 (500 Tests) • **Store at 4°C**

LSBio
LifeSpan BioSciences, Inc.

Assay Procedure

Procedure Using 96-Well Plate

Important: The reagent must be brought to room temperature and well shaken before use. The Blue Reagent is highly sensitive to phosphate. It is important that all enzyme preparations and assay buffers not contain free phosphate. Lab detergents may contain high levels of phosphate. Make sure that lab wares are washed thoroughly with distilled water and free from contaminating phosphate.

1. Dilution of phosphate standards. Prepare a 1000 µL 40 µM phosphate Premix solution by mixing 40 µL 1 mM phosphate standard with 960 µL distilled water. Number the tubes. Prepare concentration standards by diluting the Premix as shown in the Table below.

No	Premix + H_2O	Final Vol (µL)	Phosphate Conc (µM)	pmoles Phosphate in 50 µL
1	200µL + 0µL	200	40	2,000
2	160µL + 40µL	200	32	1,600
3	120µL + 80µL	200	24	1,200
4	80µL + 120µL	200	16	800
5	60µL + 140µL	200	12	600
6	40µL + 160µL	200	8	400
7	20µL + 180µL	200	4	200
8	0µL + 200µL	200	0	0

   Transfer 50 µL diluted standard in duplicate to wells in a clear-bottom 96-well plate. Store diluted standard at 4°C for future use.

2. Transfer 50 µL test sample (e.g. enzyme reaction) in duplicate into wells of the microplate. In the case of enzyme reactions, the reaction may be terminated by either adding a specific inhibitor, or can be stopped directly by the addition of the Blue Reagent. Reaction buffer can be added as a blank control for the samples.
3. Add 100 µL of the Blue Reagent to each well. Mix by tapping the plate.
4. Incubate for 30 min at room temperature for color development.
5. Measure absorbance at 620 nm (600 nm - 660nm) on a plate reader.

Procedure Using Cuvette

For cuvette assays, add 800 µL Reagent to 400 µL sample and standards. Perform the assay as described for the microplate assay.

General Considerations

Incubation time. The chromogenic reaction is completed within 30 min.

Precipitation may occur at high concentrations of phosphate (>100 µM), or in the presence of high concentrations of e.g. proteins and metals. If precipitation occurs, dilute samples in distilled water and repeat the assay.

Enzyme reaction buffer. Because any exogenous free phosphate would interfere with the assay, it is important to ensure that the protein preparation, the reaction buffer and lab wares employed in the assay should not contain free phosphate. This can be conveniently checked by adding the Reagent to the buffer and measuring the color formation.

Phosphate Assay Kit (Colorimetric)

LS-K328-500 (500 Tests) • **Store at 4°C**

Data Analysis

Plot pmoles phosphate versus OD_{620nm} for the standard curve. Use linear regression analysis to determine amount of free phosphate in the test samples.

Sample Data

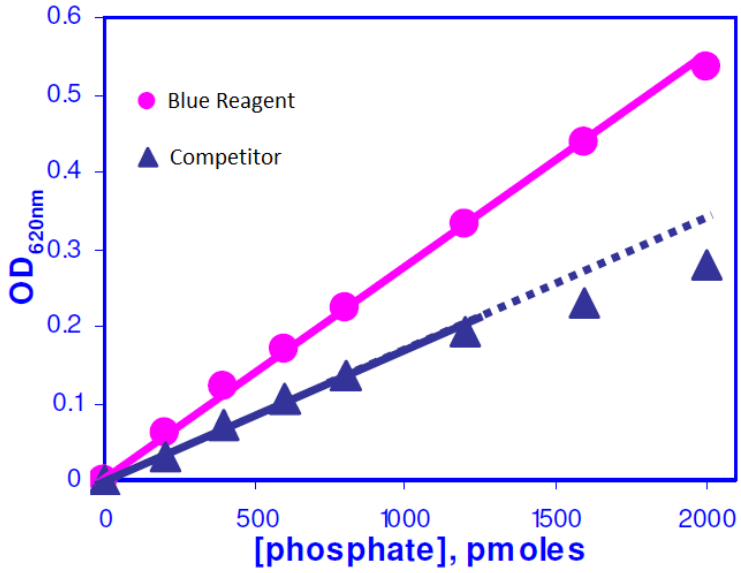

Figure 1. Phosphate standard curve in 96-well plate assay. 100 µL Blue Reagent was added to 50 µL phosphate standard. After 30 minute incubation, the OD at 620 nm was measured on a plate reader. Useful detection range was 0.4 µM to 50 µM phosphate. The detection limit estimated from blank control values was 20 pmoles. Coefficient of variance was generally below 6%. Z' factor was > 0.7. The same assay was performed using a competitor's product.

Version: V.08.09.2018

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com