Malachite Green Phosphate Assay Kit (Colorimetric)

LS-K206-2500 (2500 Tests) • **Store at 4°C**

Introduction

The Malachite Green Phosphate Assay Kit is based on quantification of the green complex formed between Malachite Green, molybdate and 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 executed in tubes, cuvettes or multi-well plates. The assays can be conveniently performed in 96- and 384-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 1.6 pmoles of phosphate and useful range between 0.02 µM and 40 µM phosphate.
- Fast and convenient: homogeneous "mix-and-measure" assay allows quantitation of free phosphate within 20 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 and 384-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	K206-2500
	2500 Tests
Reagent A	50 mL
Reagent B	1 mL
Standard (1 mM phosphate)	1mL

Storage

The kit is shipped at room temperature. The reagents and standard are stable for one year 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

Malachite Green Phosphate Assay Kit (Colorimetric)

LS-K206-2500 (2500 Tests) • **Store at 4°C**

Assay Procedure

Procedure using 96-well plate

Reagent Preparation. Each assay requires 20 µL Working Reagent. Prepare enough Working Reagent by mixing 100 vol of Reagent A and 1 vol of Reagent B (e.g. 5 mL Reagent A and 50 µL Reagent B). Working Reagent is stable for at least 1 day at room temperature.

Important: The reagent must be brought to room temperature before use. Before each assay, it is important to check that all enzyme preparations and assay buffers do not contain free phosphate. This can be conveniently done by adding 20 µL of the Working Reagent to 80 µL sample solution. The blank OD values at 620 nm should be lower than 0.2. If the OD readings are higher than 0.2, check water phosphate level. Double distilled water usually have OD readings lower than 0.1. Lab detergents may contain high levels of phosphate. Make sure that lab wares are free from contaminating phosphate after thorough washes.

1. Preparation of phosphate standards. Prepare a Premix solution containing 40 µM phosphate by pipetting 40 µL 1 mM phosphate standard to 960 µL distilled water or enzyme reaction buffer. Number the tubes.

   Dilute standards as shown in the following Table. Pipette 80 µL standard in duplicate into wells of a clear-bottom 96-well plate. Add blank controls containing water or reaction buffer only.

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

2. Transfer 80 µL test samples into separate wells of the plate.

   Note: in the case of enzyme reactions, the reaction may be terminated by adding a specific inhibitor, or can be stopped directly by the addition of the Working Reagent. Dilution of reaction mixture may be necessary prior to the assay (see General Considerations). For ATPase or GTPase assays, the ATP or GTP concentration should be lower than 0.25 mM. If the reaction mixture contains > 0.25mM ATP or GTP, dilute samples in distilled water. For example, if the ATPase reaction contained 1 mM ATP, at the end of reaction dilute reaction mixture 4-fold in water prior to the assay.

3. Add 20 µL of Working Reagent to each well. Mix gently by tapping the plate.
4. Incubate for 30 min at room temperature for color development.
5. Measure absorbance at 600 nm - 660nm (620 nm) on a plate reader.

For assays in 384-well plates, the procedures are the same, except that the volume of the standard and sample solution should be 40 µL and that of the Working Reagent should be 10 µL.

Calculation

Plot OD_{620nm} versus phosphate standard concentrations. Determine sample phosphate concentrations from the standard curve.

General Considerations

Incubation time. The chromogenic reaction is completed within 30 min at room temperature. Read OD values at 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, perform a series dilution of sample in H2O, run the assay and determine the dilution factor from wells with no precipitation. Repeat assays using diluted samples.

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 Working Reagent to the buffer and measuring the color formation.

Liquid disposal. The assay mixture contains 0.4 M sulfuric acid. It is recommended that the waste liquid be neutralized with equal volume of 1 N NaOH prior to disposal.

Sample Data

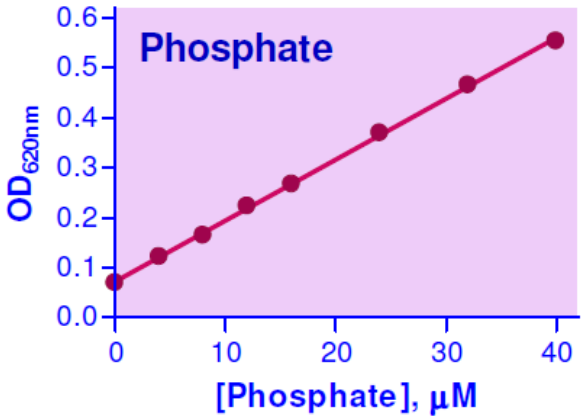

Figure. Phosphate standard curve in 96-well plate. After 30 minute incubation, the OD at 620 nm was read. Data are presented as mean ± SD (n = 2). Useful detection range was 0.02 to 40µM phosphate.

Version: V.08.09.2018