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Product Information

Phosphate Assay Kit

Catalog Number **MAK308**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
This Phosphate Assay Kit is based on a proprietary formulation of the malachite green dye. The colorimetric 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.

This kit may be used to measure the liberation of free orthophosphate in the following 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.

The non-radioactive colorimetric assay kit has 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 multiwell plates. The assays can be conveniently performed 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 0.4–50 μM phosphate.
- Fast and convenient: homogeneous “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 0.7–0.9 are observed in 96 well plates. Can be readily automated on HTS liquid handling systems.

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

Malachite Green Reagent Catalog Number MAK308A	50 mL
1 mM Phosphate Standard Catalog Number MAK308B	1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.

Precautions and Disclaimer
This product is 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.

Preparation Instructions
The components of this kit are ready to use. Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Storage/Stability
The kit is shipped at ambient temperature. Store all components at 2–8 °C upon receiving.

Procedure
All samples and standards should be run in duplicate. Use ultrapure water for the preparation of samples and standards.

Note: The Malachite Green Reagent must be brought to room temperature and well shaken before use. The Malachite Green 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 labware is washed thoroughly with ultrapure water and free from contaminating phosphate.

1. Dilution of phosphate standards. Prepare 1,000 µL of 40 µM phosphate Premix solution by mixing 40 µL of 1 mM phosphate standard with 960 µL of ultrapure water. Number the tubes. Prepare concentration standards by diluting the Premix as shown in Figure 1.

Figure 1
Preparation of Standards

#	Premix + water	Final Vol (µL)	Phosphate Conc (µM)	pmoles Phosphate in 50 µL
1	200 µL + 0 µL	200	40	2,000
2	160 µL + 40 µL	200	32	1,600
3	120 µL + 80 µL	200	24	1,200
4	80 µL + 120 µL	200	16	800
5	60 µL + 140 µL	200	12	600
6	40 µL + 160 µL	200	8	400
7	20 µL + 180 µL	200	4	200
8	0 µL + 200 µL	200	0	0

Transfer 50 µL of prepared standard in duplicate to wells in a clear-bottom 96 well plate. Store diluted standard at 2–8 °C for future use.

2. Transfer 50 µL of 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 Malachite Green Reagent. Reaction buffer can be added as a blank control for the samples.

Notes: 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 ultrapure water and repeat the assay.

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

3. Add 100 µL of the Malachite Green Reagent to each well. Mix by tapping the plate.

4. Incubate for 30 minutes at room temperature for color development.

5. Measure absorbance at 600–660 nm (620 nm) on a plate reader.

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

Results
Calculation
Plot $OD_{620\ nm}$ versus phosphate standard concentrations. Use linear regression analysis to determine amount of free phosphate in the test samples.

References

High-throughput Screening

1. Rumsfeld, J. et al., High-throughput assay for inorganic pyrophosphatases using the cytosolic enzymes of *Saccharomyces cerevisiae* and human as an example. Protein Expr. Purif., **18**(3), 303-9 (2000).
2. Cogan, E.B. et al., A robotics-based automated assay for inorganic and organic phosphates. Anal. Biochem., **271**, 29-35 (1999).
3. Ng, D.H. et al., Nonradioactive method to measure CD45 protein tyrosine phosphatase activity isolated directly from cells. J. Immunol. Methods, **179**(2), 177-85 (1995).
4. Fisher, D.K., and Higgins, T.J., A sensitive, high-volume, colorimetric assay for protein phosphatases. Pharm. Res., **11**(5), 759-63 (1994).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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