Malachite Green Inorganic Phosphate Assay Kit

Cat.No: MCA801

Size: 500 assays/ 96-well format

**Sufficient for 500 total reactions. Includes reagents required to perform standard curves.*

Introduction

Background Phosphate is an essential and abundant mineral in living organisms, playing critical roles in both physiological and pathological processes. It is widely used as a fertilizer in agriculture and serves as an environmental indicator of water eutrophication. In the food industry, phosphate is a common additive in processed foods and beverages, although excessive intake may pose health risks.

The **Malachite Green Phosphate Assay** is a sensitive, cost-effective, and straightforward colorimetric method for phosphate quantification. It is widely used in life sciences to measure enzyme activities such as phosphatases and kinases and to screen for inhibitors. In addition, this assay is applied in environmental monitoring, agriculture testing, food quality control, and clinical diagnostics, to assess inorganic phosphate levels in water, soil, food products, and biological samples.

Principle Malachite Green is a basic dye belonging to the triphenylmethane group. This assay is based on the formation of a green-colored complex between malachite green, ammonium molybdate, and free orthophosphate under acidic conditions. In this reaction, inorganic orthophosphate combines with molybdate to form a phosphomolybdate complex, which subsequently binds to the malachite green molecule, resulting in a major shift in absorption. The intensity of the resulting green color is directly proportional to the concentration of inorganic phosphate in the sample and can be quantified between **600–650 nm** using a spectrophotometer or microplate reader.

This assay measures only **free inorganic phosphate**. Lipid-bound or protein-bound phosphates must be completely hydrolyzed and neutralized prior to analysis. While this assay is optimized for **96-well plates**, the protocol can be easily scaled for alternative plate formats or cuvettes.

Application For the quantitative detection of inorganic free phosphate concentrations in serum, plasma, urine, cell lysates, cell culture supernatants, tissue/ food/ beverage homogenates, other biological fluids, water samples and soil extracts.

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Note: Turbid, lipid-rich, or highly colored samples may require prior clarification (e.g., filtration or high-speed centrifugation) to eliminate optical interference.

Safety Data and Handling This product should be handled as a potential health hazard. Appropriate precautions should be taken when handling them in a laboratory setting. Avoid ingestion, inhalation, and contact with eyes, skin, or clothing. Wash thoroughly after handling them.

For comprehensive hazard identification, first aid measures, and disposal considerations, users should refer to the **Safety Data Sheet (SDS)** prior to use.

FOR RESEARCH USE ONLY

Not for Diagnostic or Therapeutic Use

PRODUCT DATASHEET

Malachite Green Inorganic Phosphate Assay Kit

Ver. 1.0

Catalog no. **MCA801**

Content & Storage

Kit Components

Part. No.	Item	Concentration	Description	Volume	Storage
MCA801A	**Phosphate Standard**	50 μM	Phosphate Standard	5 mL	RT
MCA801B	**Color Reagent**	-	Malachite Green Coloring Reagent	25 mL	4°C protected from light

Additional Materials Required (not included)

Ultrapure water (phosphate-free)

Plate reader or spectrophotometer (absorbance 600-650 nm)

96-well plate or spectrophotometer cuvette

Procedures

Assay procedures

For 96-well plates, each well requires a total reaction volume of **200 μL**, consisting of 160 μL sample and 40 μL Color Reagent. Mix at a **4:1** ratio (sample: Color Reagent).

***Note**: This assay is compatible with other multiwell plates or cuvettes, provided the 4:1 volume ratio remains unchanged.*

Sample preparation

Liquid samples, including urine and serum, can be used directly.

Solid samples, such as food or tissue, should be homogenized, centrifuged, and appropriately diluted before utilizing the supernatant for analysis.

Prepare a minimum sample volume of **160 μL per well**. It is highly recommended to prepare an extra 10–20% volume to ensure accurate pipetting.

Phosphate Standard preparation

This kit supports a linear phosphate detection range of **0–8 nmol** per well.

Prepare standards by diluting the 50 μM **Phosphate Standard** according to the table below.

To ensure accurate pipetting without running out of volume, the table below provides a total preparation volume of 200 µL per tube.

Standard	Final Pi Concentration (µM)	Volume of 50 µM Phosphate Standard (µL)	Volume of Diluent (µL)	Absolute Pi per well (nmol)*
S1	0 (blank)	**0**	**200**	**0.0**
S2	10	**40**	**160**	**1.6**
S3	20	**80**	**120**	**3.2**
S4	30	**120**	**80**	**4.8**
S5	40	**160**	**40**	**6.4**
S6	50	**200**	**0**	**8.0**

Notes:

- *Absolute Pi per well is based on loading exactly **160 µL** of the prepared standard into the well. For optimal linearity of the phosphate standard curve, prepare dilutions immediately before use.*
- *Use phosphate-free ultrapure water (or an appropriate buffer) for dilution.*
- *Additional concentration can be prepared using the same dilution principle if required.*

Phosphate quantification protocol

1. Add **160 µL** of ultrapure water (blank), prepared **Phosphate Standards**, and experimental samples to their designated well.
2. Add **40 µL** of **Color Reagent** to the corresponding wells.
3. Gently tap or shake the plate to mix the contents. Incubate at room temperature for 30 minutes.
4. Measure the optical density (OD) of each well at **640 nm** (or alternative wavelength between 600–650 nm) using a microplate reader or spectrophotometer.

Notes:

- ***Loading order:** Load standard from **lowest to highest concentration** to minimize time-dependent color development variations across the plate*
- ***No centrifugation**: Do not centrifuge the well or reaction tubes after adding the Color Reagent. Centrifugation can precipitate the green complex particles, which can affect the OD measurement.*

Analysis and Calculation

Subtract the blank absorbance (S1) from all phosphate standards and experimental samples OD values.

Plot the blank-corrected absorbance (ΔOD_{640}) on the Y-axis versus the absolute phosphate amount (nmol) on the X-axis. The linear regression should achieve an R^2 value ≥ 0.99.

Determine the phosphate concentration of your samples using the linear standard curve slope equation:

$$C_{sample} = \frac{OD_{sample} - OD_{blank}}{slope}$$

The Phosphate Standard Curve

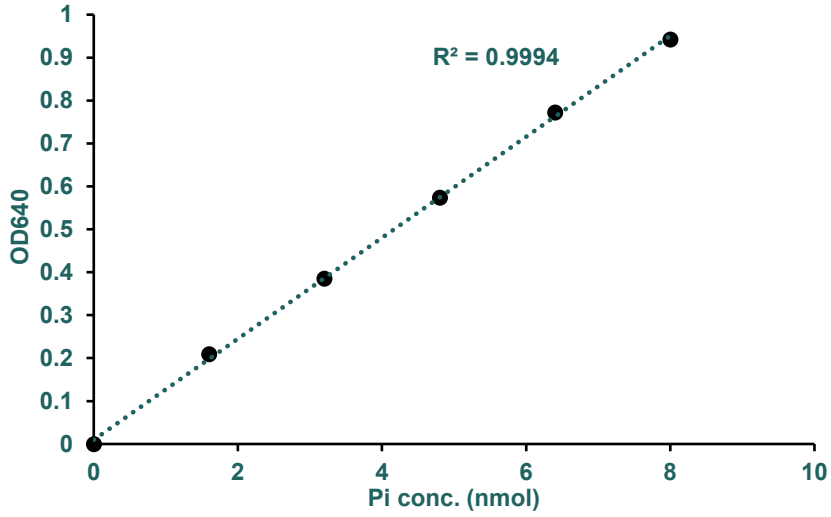


Figure 1: Reference phosphate standard curve of this kit

Troubleshooting

For troubleshooting, please visit our site: www.tri-rbiotech.com.tw

Contact Us

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References

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