Malachite Green Phosphate Assay Kit

Catalog No.: abx298878

Size: 100 Assays

Storage: Store all components in the dark at 4°C.

Application: For quantitative detection of Inorganic free phosphate concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 1 µmol/L – 50 µmol/L

Introduction: Malachite Green Phosphate Assay Kit provides a fast, reproducible, and non-radioactive method for measuring inorganic free phosphate in aqueous solutions. This simple assay method is based on the complex formed between malachite green molybdate and free orthophosphate under acidic conditions. The formation of the green molybdophosphoric acid complex measured at 635 nm is directly related to the free organic phosphate concentration. Applications for this assay include quantification of phosphorylation and phosphate release from protein phosphatase substrates. This assay measures only inorganic free phosphate; lipid-bound or protein-bound phosphates must first be hydrolyzed and neutralized prior to measurement.

Abbexa's Malachite Green Phosphate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating inorganic free phosphate concentrations. The dye reagents react with Phosphate to create an absorption maximum at 635 nm. The intensity of the color is proportional to the concentration of Phosphate, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Dye Reagent A: 4 mL
4. Dye Reagent B: 1 vial
5. Dye Reagent B Diluent: 6 ml
6. 50 µmol/L Standard: 1 mL

Materials Required But Not Provided

1. Microplate reader (635 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar
9. Water bath

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent B Solution**
  Add 6 mL of Dye Reagent B Diluent into the Dye Reagent B vial and mix thoroughly to prepare the Dye Reagent B Solution. Warm the vial using a water bath until the powder has dissolved.

2. Sample

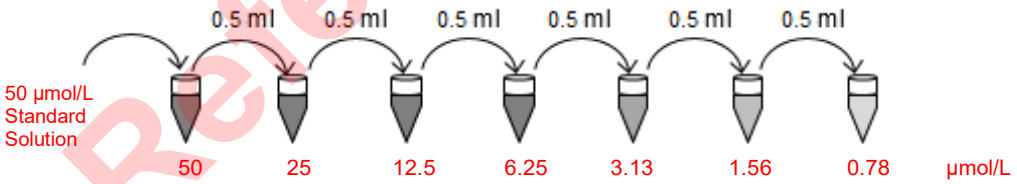
- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 mL of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 12,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 mL of Assay Buffer on ice. Centrifuge at 12,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.
- **Liquid, plasma and serum samples**
  Liquid samples can be used directly.

B. Assay Procedure

Warm all reagents to 37°C prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 50 µmol/L, 25 µmol/L, 12.5 µmol/L, 6.25 µmol/L, 3.13 µmol/L, 1.56 µmol/L, and 0.78 µmol/L. Aliquot 0.5 mL of distilled water into each tube. Add 0.5 mL of 50 µmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 mL from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 40 µL of Dye Reagent A Solution to all wells.
4. Add 60 µL of Dye Reagent B Solution to all wells.
5. Tap the plate gently to mix. Allow to stand for 10 minutes.
6. Add 100 µL of sample to the sample wells.
7. Add 100 µL of prepared standards to the standard wells.
8. Add 100 µL of distilled water to the blank wells.
9. Tap the plate gently to mix. Allow to stand for 5 minutes. Read and record absorbance at 635 nm.

C. Calculations

Inorganic free phosphate concentration per mg of protein:

$$\textbf{Phosphate}\ (\mu\textbf{mol/mg}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{0.05}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Inorganic free phosphate concentration per g of sample:

$$\textbf{Phosphate}\ (\mu\textbf{mol/g}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{0.05}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Inorganic free phosphate concentration per 10^4 cells or bacteria:

$$\textbf{Phosphate}\ (\mu\textbf{mol}/\mathbf{10^4}\ \textbf{cells}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{N}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{0.05}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Inorganic free phosphate concentration per mL of sample:

$$\textbf{Phosphate}\ (\mu\textbf{mol/mL}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{0.05} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/mL)
$\mathbf{C_{Standard}}$	Concentration of highest standard (50 µmol/L= 0.05 µmol/mL)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer (1 mL)
$\mathbf{V_{Sample}}$	Volume of sample (0.05 mL)
$\mathbf{V_{Standard}}$	Volume of standard (0.05 mL)