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 samples, and other biological fluids.

Detection Range: 0.001 mmol/L – 0.2 mmol/L

Sensitivity: 0.001 mmol/L

Introduction: Abbexa's Malachite Green Phosphate Assay Kit is a fast, reproducible, and non-radioactive assay for measuring inorganic free phosphate in aqueous solutions, based on the complex formed between malachite green molybdate and free orthophosphate under acidic conditions, producing a green molybdophosphoric acid complex with an absorption maxima at 636 nm. The intensity of the color is directly proportional to the free organic phosphate concentration, which can then be calculated.

This kit is suitable for 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.

Kit components

1. 96 well microplate
2. Assay Buffer: 60 ml
3. Dye Reagent 1: 12 ml
4. Dye Reagent 2: 4 ml
5. Standard (10 mmol/L): 1 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (636 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Double distilled water
5. Normal Saline (0.9% NaCl)
6. Timer
7. Ice
8. Sonicator
9. Mortar
10. Water bath

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent 1 Solution**

  Warm the vial using a water bath until the gel has completely dissolved.

- **Detection Working Solution**

  Mix Dye Reagent 1, Dye Reagent 2 and double distilled water at a ratio of 3:1:4. For example, add 12 ml of Dye Reagent 1, 4 ml of Dye Reagent 2, and 16 ml of Double distilled water. Mix thoroughly and incubate at 37°C for 1 hour before assay. Prepare the required volume immediately before use, and use within the same day.

- **Standard Solution (0.2 mmol/L)**

  Dilute the 10 mmol/L Standard 50-fold with double distilled water. For example, dilute 1 ml of 10 mmol/L Standard with 49 ml double distilled water to prepare 50 ml of Standard Solution (0.2 mmol/L). The prepared solution can be stored at 4°C for up to 7 days.

2. Sample

- **Cell samples**

  Count the number of cells in the culture. Collect the cells into a centrifuge tube, wash the cells twice with Normal Saline, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Normal Saline 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. Dilute the supernatant with Assay Buffer 2-fold. 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**

  Accurately weigh the sample. For every 0.1 g of sample, homogenize in in 0.9 ml of Normal Saline on ice. Centrifuge at 12,000 × g at 4°C for 10 minutes. Dilute the supernatant with Assay Buffer 2-fold. Centrifuge at 12,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

- **Serum/Plasma samples**

  Dilute the sample with Assay Buffer 2-fold. Centrifuge at 12,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

Recommended dilution ratios

Samples should be diluted with Normal Saline (0.9% NaCl). When the absolute OD value of a sample is above 1.2, we would recommend increasing the dilution ratio.

Sample type	Dilution ratio
10% Rat liver tissue homogenate	15-25
10% Rat heart tissue homogenate	15-25
10% Rat spleen tissue homogenate	15-25
10% Rat brain tissue homogenate	15-25
10% Rat kidney tissue homogenate	15-25
10% Mouse heart tissue homogenate	15-25
10% Mouse lung tissue homogenate	15-25
10% Mouse ovarian tissue homogenate	50-70
Rat serum	8-12
Rat plasma	8-12
Human plasma	8-12
HL-40 cell (1,000,000 cells)	2-5

Note:

This assay is particularly sensitive to contamination by Phosphorous. Wash all equipment thoroughly before use. **Do not use PBS as sample diluent.**

B. Assay Procedure

Bring all reagents to room temperature 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 0.20 mmol/L, 0.15 mmol/L, 0.12 mmol/L, 0.10 mmol/L, 0.05 mmol/L, 0.02 mmol/L, and 0.01 mmol/L. Double distilled water serves as the control (zero) well. Prepare the standard curve according to the following table.

Volume of 0.2 mmol/L Standard Solution (µl)	Volume of double distilled water (µl)	Standard Concentration (mmol/L)
200	0	0.20
150	50	0.15
120	80	0.12
100	100	0.10
50	150	0.05
20	180	0.02
10	190	0.01
0	200	0

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 20 µl of prepared standards to the standard wells.
4. Add 20 µl of sample to the sample wells.
5. Add 200 µl of Detection Working Solution to each well. Mix fully and incubate at 37°C for 20 minutes in the dark
6. Read and record absorbance at 636 nm.

C. Calculations

The standard curve can be plotted as the absolute absorbance of each standard solution (y) vs. the respective concentration (y). A linear fit is recommended for the standard curve (y = ax + b).

Inorganic free phosphate concentration per kg of wet sample:

$$\mathbf{Phosphate\ (mmol/kg)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}) - \mathrm{b}}{\mathrm{a}} \times \frac{\boldsymbol{f} \times \mathbf{2} \times \mathbf{V}}{\mathbf{m}}$$

Inorganic free phosphate concentration per 10^6 cells or bacteria:

$$\mathbf{Phosphate\ (mmol/10^6\ cells)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}) - \mathrm{b}}{\mathrm{a}} \times \frac{\boldsymbol{f} \times \mathbf{2} \times \mathbf{V}}{\mathbf{N}}$$

Inorganic free phosphate concentration per L of sample:

$$\mathbf{Phosphate\ (mmol/L)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}) - \mathrm{b}}{\mathrm{a}} \times \boldsymbol{f} \times \mathbf{2}$$

where:

a	Gradient of the standard curve (y = ax + b)
b	Intercept of the standard curve (y = ax + b)
m	Mass of the sample (g)
N	Number of cells or bacteria (× 10^6)
V	Volume of normal saline used in sample preparation (ml)
f	Dilution factor prior to assay
2	Dilution factor in sample preparation