Malachite Green Phosphate Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6719

Detection and Quantification of Phosphate content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

For research use only . Not for diagnostic or therapeutic procedures .

1. Introduction

Malachite Green Phosphate Colorimetric Microplate As say 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. Overall, this assay is a reliable and suitable means of detecting and quantifying mi nimal amounts of inorganic free phosphate in acidic environments and is amenable to high-throughput screening applications.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30 mlx 4	4 °C
Dye Reagent I	4 mlx 1	4 °C, keep in dark
Dye Reagent II	Powder x 1	4 °C
Dye Reagent II Diluent	6 mlx 1	4 °C
Standard (50 μmol/L)	1 mL x 1	4 °C
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 635 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening.Perform 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve.The concentration of standard curve could be 50/25/12.5/6.25/3.12/1.56/0.78 μmol/L.

Dye Reagent II: Add 6 mL Dye Reagent II Diluent and heat to dissolve before use. Store at 4°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. Sample Preparation

1. For cell and bacteria samples Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL Assay Buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s ,repeat 30 times); centrifuged at 10,000g 4°C for 10minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples Weigh out 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice, centrifuged at 10,000g 4°C for 1 0minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples Detect directly.

6. Assay Procedure

Add following reagents in to the microplate :

Reagent*	Sample**	Standard	Blank
Dye Reagentl	40 μl	40 μl	40 μl
Dye Reagent II	60 μl	60 μl	60 μl
Mix.			
Sample	100μl	--	
Standard	--	100 μl	--
Distilled water	--	--	100μl
Mix,wait for 2 minutes, measure at 635 nm and recordthe absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (\mu mol/L)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

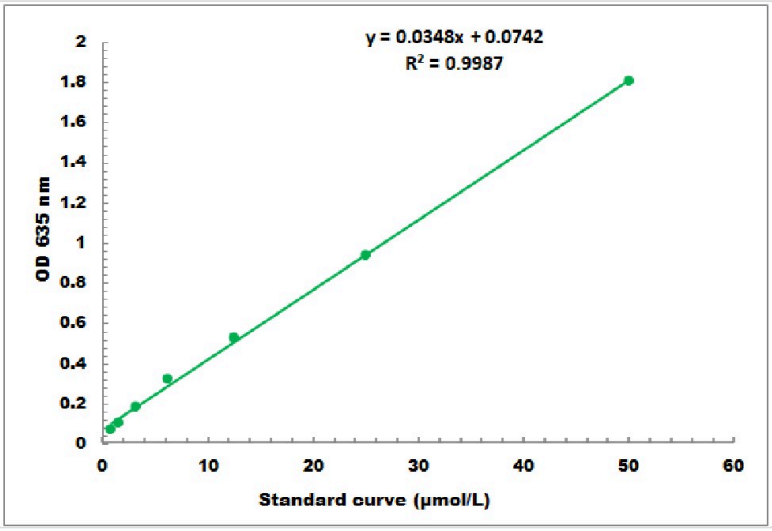
2.4According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \ (\mu mol/ml)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, μmol/ L
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl
V_{Assay}	the volume of Assay Buffer, μl
$C_{Protein}$	the sample protein concentration, mg/ml
W	the weight of sample, g
N	the quantity of cell or bacteria, 10^4

8. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range : 0.5μmol/L - 50 μmol/L