Malachite Green Phosphate Assay Kit

User Manual

Catalog # FTA0110

(Version 1.2C)

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

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

I. 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. Overall, this assay is a reliable and suitable means of detecting and quantifying minimal amounts of inorganic free phosphate in acidic environments and is amenable to high-throughput screening applications.

II. KIT COMPONENTS

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

Dye Reagent II: add 6 ml Dye Reagent II Diluent and heat to dissolve before use, store at 4 °C.

III. 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

IV. 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%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 10 minutes, 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 12,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Dye Reagent I	40 µl	40 µl	40 µl
Dye Reagent II	60 µl	60 µl	60 µl
Mix.			
Sample	100 µl	--	
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Mix, wait for 2 minutes, measured at 635 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Phosphate (μmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Phosphate (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Phosphate (μmol/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{Phosphate (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 0.05 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the protein concentration, 50 μmol/L = 0.05 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

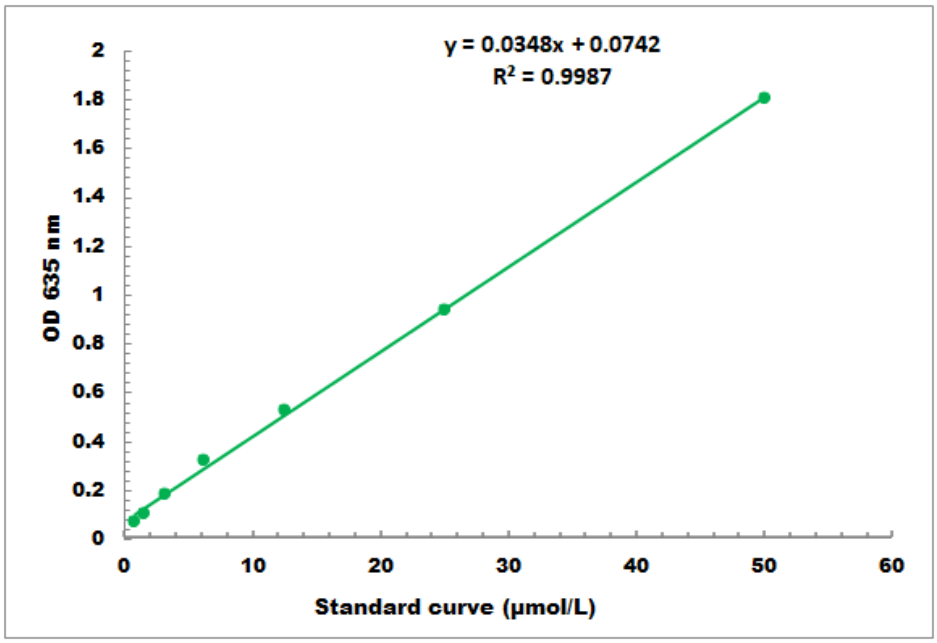
$V_{Standard}$: the total volume of the reaction, 0.1 ml;

V_{Sample}: the volume of sample, 0.1 ml;

V_{Assay}: the volume of assay buffer, 1 ml.

VII. TYPICAL DATA

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



Detection Range: 1 μmol/L - 50 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES