Malachite Green Phosphate Assay

Cat. No. 8118
2500 tests

Introduction

Phosphorylation and dephosphorylation, which are the addition and removal of phosphate (PO_4^{3-}) groups to and from protein molecules respectively, are important regulatory mechanisms involved in cell-cycle regulation and signal transduction. ScienCell™ Malachite Green Phosphate Assay provides a simple colorimetric method for the determination of soluble inorganic phosphate concentrations based on the complexation of malachite green oxalate with phosphomolybdate under acidic conditions. Applications of the kit include measurement of phosphate release, quantification of protein- or lipid- phosphorylation, etc. For quantification of protein or lipid bound phosphate, the phosphorylated proteins or lipids need to be hydrolyzed and neutralized before phosphate measurements are performed.

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8118a	1	10 mM Phosphate Standard	0.25 ml	4ºC
8118b	1	Malachite Green Reagent A	437.5 mg	4ºC
8118c	1	Malachite Green Reagent B	25 ml	4ºC

Additional Materials Required (not included)

3M sulfuric acid

Product Use

This assay kit is used to evaluate phosphate level *in vitro*. It is for research use only. Not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from ScienCell™ Malachite Green Phosphate Assay of phosphate solutions with concentrations ranging from 1.5625 to 37.5 µM show a linear relationship between OD_{630nm} and phosphate concentration (Figure 1).

Procedures

A. Preparation of Malachite Green Reagent A:

1. Dissolve 437.5 mg of Malachite Green Reagent A in 25 ml of 3M sulfuric acid to make a 1.75% solution.

B. Preparation of phosphate standards:

1. Prepare a 50 µM phosphate standard by adding 5 µl of 10 mM Phosphate Standard to 995 µl of DI H_2O.
2. Prepare a phosphate standard curve using the 50 µM phosphate standard according to Table 1. For each point, 160 µl of diluted phosphate standard is prepared to provide three replicates of 50 µl.

C. Assay procedure:

1. Add 50 µl of phosphate standard, sample or blank into each well of 96 well plate.
2. Add 10 µl of Malachite Green Reagent A to each well, mix and incubate for 10 minutes at room temperature.

3. Add 10 µl of Malachite Green Reagent B to each well, mix and incubate for 20 minutes at room temperature.
4. Read absorbance at 630 nm on a plate reader.

D. Calculations:

1. Average the OD_{630nm} of replicate wells of each phosphate standard, sample and blank. Subtract the average OD_{630nm} value of the blank from the average OD_{630nm} values obtained with all other samples.
2. Based on the calibrated OD_{630nm} of the phosphate standard, make a standard curve by plotting OD_{630nm} as a function of phosphate concentration. (See Figure 1 for a typical standard curve.) Determine the equation and R^2 value of the trend line.
3. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the phosphate concentration of samples as follows:

$$[PO_4{}^{3-}] = \frac{OD_{630nm} - B}{A}$$

No.	50 µM phosphate (µL)	DI H_2O (µL)	Phosphate concentration (µM)
1	160	0	50
2	80	80	25
3	40	120	12.5
4	20	140	6.25
5	10	150	3.125
6	5	155	1.5625
7	0	160	0 (Blank)

Table 1. Preparation of phosphate standards in Malachite Green Phosphate Assay.

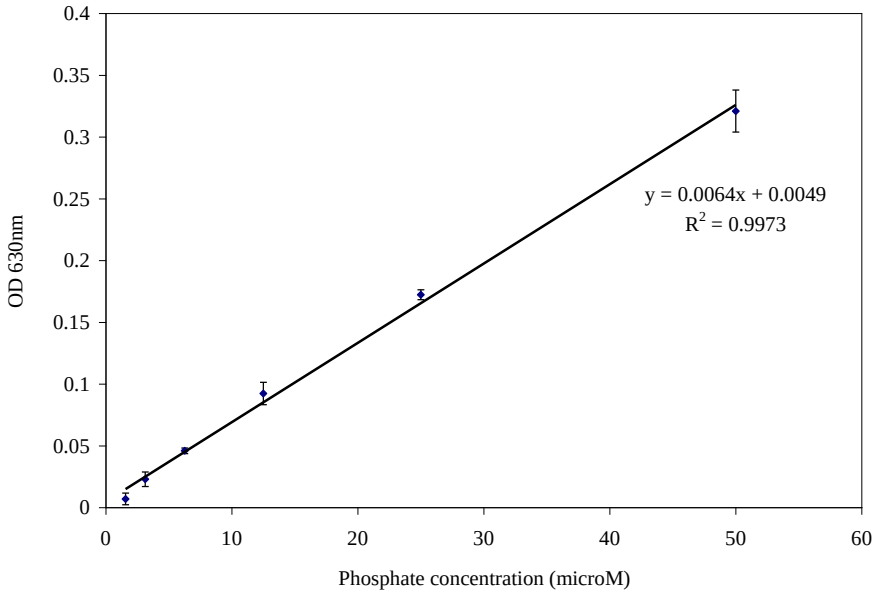

Figure 1. A typical phosphate standard curve measured by ScienCell™ Malachite Green Phosphate Assay.