MA-0105

Phosphate Assay

(500 wells, Colorimetric, OD 650 nm, Store at room temperature)

Background Information:

Phosphate is found virtually everywhere in biological systems. It functions in a variety of roles, both structural and functional. One of the most important roles is as a molecular switch. There are over 500 kinases and 200 phosphatases in the human genome alone which function by adding or removing phosphate groups to/from hundreds or thousands of molecules including proteins, sugars, nucleic acids, lipids and a plethora of other small molecules. AkrivisBio's Phosphate Assay utilizes a classic formulation of malachite green and ammonium molybdate originally described by Itaya and Ui in 1965, in which a chromogenic complex is formed with phosphate ion giving an intense absorption band around 650 nm. Phosphate is readily quantifiable at concentrations as low as 1 µM.

Assay Principle:

1 – Phosphate at low pH in the presence of molybdate forms phosphomolybdate
2 – Malachite green forms a chromogenic complex with phosphomolybdate

Assay Components:

Phosphate Reagent	15 ml	WM	MA-0105A
Phosphate Standard (10 mM)	0.5 ml	Yellow	MA-0105B

**CAUTION – Phosphate reagent contains sulfuric acid and should be handled with care.

Assay Protocol:

1. Phosphate Standard Curve:

Add 10 µl of the 10 mM Phosphate Standard to 990 µl DI H_2O to make 100 µM Phosphate Standard. Add 0 – 10 – 20 – 30 – 40 – 50 µl of the diluted Phosphate Standard to a series of wells of a 96-well plate. Adjust the volume to 200 µl with DI H_2O to generate 0 – 1 – 2 – 3 – 4 - 5 nmol of Phosphate standard.

2. Preparation of sample:

No sample pretreatment is necessary. For biological samples, homogenize tissue (10 mg), cells (10^6) with DI water. Centrifuge at 16,000 X g or use a centrifugal filter to remove insoluble materials. Transfer clear extract to a fresh tube. Transfer 0-200 µl of clear liquid samples to a 96-well plate and adjust the volume of all wells to 200 µl with DI H_2O.

Note: Many laboratory detergents contain high amounts of phosphates which can adhere to cleaned glassware. Use only new unused disposable plastic labware for all samples, standards and reagents to avoid contamination.

3. Reaction:

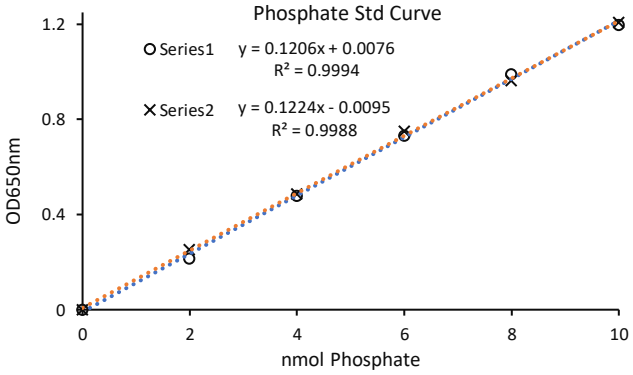
Add 30 µl Phosphate Reagent to all standard and sample wells, and mix. Cover the plate and allow for full color development at RT for 30 min.

4. Measurement:

Read the absorbance at 650 nm using a plate reader. The color is stable for several hrs.

5. Typical Result

nmole Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0889	0.0902	0	0
2	0.3039	0.3427	0.2150	0.2525
4	0.5692	0.5785	0.4803	0.4883
6	0.8209	0.8413	0.7320	0.7511
8	1.0795	1.0532	0.9906	0.9630
10	1.2874	1.3004	1.1985	1.2102



6. Calculations:

Subtract the 0 nmol Phosphate reading from all other standard and test sample readings. Plot the Phosphate Standard Curve. Determine the slope of the Standard Curve. The slope of the Standard Curve defines the system sensitivity of the assay in OD/nmol Phosphate. Divide the background corrected sample readings by the slope of the Standard Curve to determine the amount of phosphate (nmol) in the sample wells. To convert back to phosphate content of samples:

A. Divide the nmoles phosphate per well by the volume of sample added to the well in µl = nmoles phosphate / µl sample.
B. Multiply the nmoles phosphate/µl sample by the total volume of liquid recovered after sample preparation = total nmole phosphate/sample.
C. Divide the total nmoles of phosphate/sample by mg tissue or # of cells used, etc. = nmole/mg sample (or per # of cells, etc.)

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48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com