

**ProFoldin**

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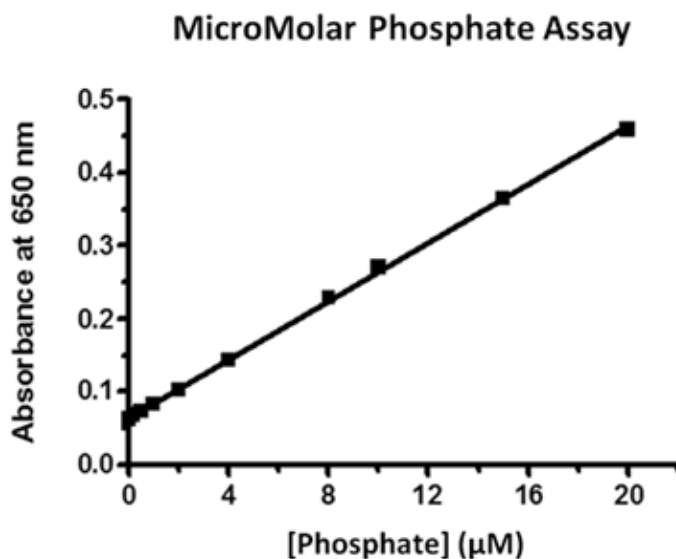
INSTRUCTIONS

ProFoldin MicroMolar Phosphate Assay Kit

CATALOG NUMBER **MPA3000**

INTRODUCTION

The MicroMolar Phosphate Assay Reagent is for measurement of 0.2 μM – 20 μM (micromolar) phosphate. The assay is based on the principle that phosphate interacts with molybdate and forms a blue complex with Malachite Green dye in an acidic solution. The phosphate amount is measured by detection of the light absorbance at 650 nm.



The assay reagent (catalog number MPA3000) includes 450 ml of reagent. It is for 3000 to 5000 assays using 96-well plates or more than 10,000 assays using 384-well plates. It can also be used for measurement of phosphate concentrations using cuvettes and a spectrophotometer.

PROTOCOLS

MEASUREMENT OF PHOSPHATE USING 96-WELL PLATES

Phosphate Standard Curve

1. Prepare standard phosphate solutions with a series of concentrations from 0.2 μM to 20 μM .
 2. Mix 100 μl of the phosphate solution with 150 μl of the Assay Reagent in the wells of a 96-well plate for 5 min and read the light absorbance at 650 nm (A_{650}).
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Data Analysis

Plot the A_{650} and the phosphate concentration [**phosphate**] to generate the linear standard curve.

$$A_{650} = a [\text{phosphate}] + b$$

Where the A_{650} values are from experimental data, the **a** and **b** values are from the linear fitting between the A_{650} values and the phosphate concentrations.

UNKNOWN SAMPLES

Follow the same procedure to measure the A_{650} values from the unknown samples. Calculate the phosphate concentrations in the unknown samples using the A_{650} values from the unknown samples and the **a** and **b** values from the standard curve.

$$[\text{Phosphate}] = (A_{650} - b) / a$$

MEASUREMENT OF PHOSPHATE USING CUVETTES

The sample and reagent volumes are based on measurements using 1 ml-cuvettes.

1. Mix 0.400 ml of a standard phosphate solution with 0.600 ml of the Assay Reagent in a 1-ml cuvette for 5 min and read the light absorbance at 650 nm. Use standard phosphate solutions with a series of concentrations from 0.2 μM to 20 μM for the standard curve.
2. Mix 0.400 ml of the sample solution with 0.600 ml of the Assay Reagent for 5 min and read the light absorbance at 650 nm. Calculate the phosphate concentration based on the standard curve.