ProFoldin
10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Tel: (508) 735-2539 FAX: (508) 845-9258
www.profoldin.com
info@profoldin.com

INSTRUCTIONS

ProFoldin

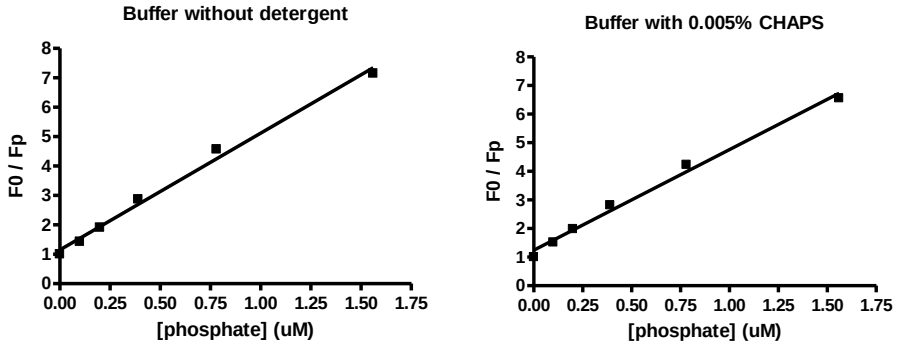
NanoMolar Phosphate Assay Kit

CATALOG NUMBER NPA1000

INTRODUCTION

The NanoMolar Phosphate Assay Kit is for measurement of sub-micromolar (< 1 μM) concentrations of phosphate. The assay is based on reduction of the fluorescence intensity of the kit reagent by phosphate. It can be used for measurement of ATPase or GTPase activity of protein samples. It is suitable for high throughput screen (HTS) of drug targets with ATPase or GTPase activity. Since the assay is ultra-sensitive to phosphate, it is particularly useful to detect ATPase or GTPase activity where a limited level of phosphate is produced. The ATP or GTP concentration used for the assay should be as low as possible because ATP and GTP products contain some phosphate level. The ATP or GTP concentration should be below 50 μM.

The assay is compatible with regular buffers with various concentrations of salts, glycerol (<5%), MgCl2, EDTA, Ethanol, DMSO and 0.005% CHAPS. Most detergents reduce the assay sensitivity. It is not compatible with DTT. TCEP-HCl (< 2 mM) instead of DTT may be used if a reducing reagent is necessary for an enzyme reaction (TCEP-HCl, Thermo Scientific, catalog number 20490). The assay is compatible with 96-well plates (Costar 3915 and Greiner 655076), 384-well plates (Corning 3571, Matrix 4318) and low volume 384-well plates (Matrix 4363). It is not compatible with some plates such as Corning 3676 or Corning 3575.



F_0 = Fluorescence intensity without phosphate; F_p = Fluorescence intensity with phosphate

The kit (Catalog number NPA1000) includes 100 ml of Reagent P1 and 1 ml of 100 x Reagent P2 and 0.1 ml of 1 mM potassium phosphate (KH_2PO_4). It is for 1000 assays using 96-well plates (100 μl of sample volume) or 4000 assays using 384-well plates (25 μl of sample volume). The following protocol is for assays using 96-well plates. Cuvettes may also be used for the measurement.

ProFoldin
10 Technology Drive, Suite 40, Number 188
Hudson, MA 01749-2791 USA
Tel: (508) 735-2539 FAX: (508) 845-9258
www.profoldin.com
info@profoldin.com

INSTRUCTIONS

PROTOCOL

Phosphate standard curve

1. Phosphate Samples: Prepare standard phosphate solutions with a series of concentrations ranging from 10 μM to zero.
2. Detection solution: For 10 assays, mix 1 ml of Reagent P1 with 0.010 ml of 100 x Reagent P2. Increase the volumes proportionally as needed.
3. Detection: Mix 100 μl of the sample with 100 μl of the detection solution for 2 min. Read the fluorescence intensity ($\mathbf{F_p}$) at 535 nm (excitation 485 nm). The fluorescence without phosphate is $\mathbf{F_o}$.
4. Data Analysis: Calculate the $\mathbf{F_o / F_p}$ ratio values and plot the correlation between the **Fo/ Fp** ratio values and the phosphate concentrations to generate the linear standard curve.

$$\mathbf{F_o / F_p = a\ [Phosphate] + b}$$

Where the $\mathbf{F_o / F_p}$ values are from experimental data, the **a** and **b** values are from the linear fitting between the $\mathbf{F_o / F_p}$ values and the phosphate concentrations.

Unknown samples

Follow the same procedure to get the $\mathbf{F_o / F_p}$ ratio values from the unknown samples. Calculate the phosphate concentrations in the unknown samples using the $\mathbf{F_o / F_p}$ ratio values from the unknown samples and the **a** and **b** values from the standard curve.

$$\mathbf{[Phosphate] = (F_o / F_p - b) / a}$$

ATPase assay protocol

The following buffer is recommended for enzyme assays: 20 mM HEPES, pH 7.5, 50 mM NaCl, 5 mM $MgCl_2$. If detergent and reducing reagent are required for the enzyme assay, 0.005% CHAPS, 1 mM TCEP may be included in the assay buffer.

1. Set ATPase reactions in a volume of 100 μl in a black 96-well plate. The ATP concentration should be less than 50 μM to avoid a high background.
2. For 10 assays, prepare the detection solution by mixing 1 ml of Reagent P1 with 0.01 ml of 100 x Reagent P2. Increase the volumes proportionally as needed.
3. Mix 100 μl of the detection solution with 100 μl of the reaction solution for 2 min and read the fluorescence intensity at 535 nm (excitation 485 nm).

   Note: The fluorescence signal should be read in 2 min after mixing the detection solution with the sample. Longer incubation time results in hydrolysis of ATP or GTP that produces phosphate background.