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INSTRUCTIONS

ProFoldin NanoMolar Phosphate Assay Kit

CATALOG NUMBER NPA1000

INTRODUCTION

The NanoMolar Phosphate Assay Kit is for measurement of sub-micromolar ($< 1 \mu\text{M}$) concentrations of phosphate. It can be used for measurement of low 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, the ATP or GTP used for an ATPase or GTPase assay should have a low background of phosphate. Typically, the ATP concentration should not be higher than $50 \mu\text{M}$. The assay is based on reduction of the fluorescence intensity of the kit reagent by phosphate.

The kit (catalog number NPA1000) provides the reagents for measurement of 1000 samples using 96-well plates (100 μl of sample volume) or 3000 to 4000 samples using 384-well plates (25 to 30 μl of sample volume). The following protocol is for assays using 96-well plates. The kit reagent can also be used for detection of phosphate concentrations using cuvettes. Adjust the volumes of the samples and the reagents proportionally for assays using 384-well plates or cuvettes.

PROTOCOL

Phosphate standard curve

1. Prepare standard phosphate solutions with a series of concentrations ranging from $10 \mu\text{M}$ to zero.
2. Mix 100 μl of the phosphate solution with 100 μl of the Reagent P1 in the wells of a 96-well black plate for 1 to 2 min. Add 100 μl of 1x Reagent P2 (diluted from the 100 x Reagent P2 with water 100-fold). Mix the solution for 2 to 5 min.
3. Read fluorescence intensity at 585 nm (excitation 485 nm) and plot the standard curve.

Phosphate detection in the absence of ATP

1. Mix 100 μl of the sample with 100 μl of the Reagent P1 in the wells of a 96-well black plate for 1 to 2 min. Add 100 μl of 1x Reagent P2 (diluted from the 100 x Reagent P2 with water 100-fold). Mix the solution for 2 to 5 min.
2. Read fluorescence intensity at 585 nm (excitation 485 nm) and use the standard curve calculate the phosphate concentration in the sample.

ATPase assay protocol

1. Set ATPase reactions in a volume of 100 μl in a black 96-well plate. The ATP concentration should be less than $50 \mu\text{M}$ to avoid a high background.
2. Dilute the 100 x Reagent P2 with Reagent P1 to make 1 x reagent. Mix 100 μl of the 1 x reagent with the 100 μl of the reaction solution for 2 to 4 min.
3. Read the fluorescence intensity at 585 nm (excitation 485 nm).