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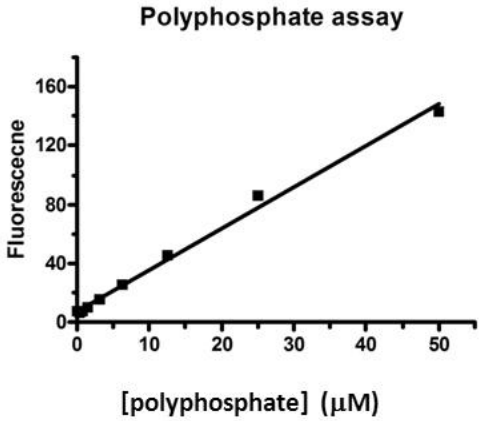
INSTRUCTIONS

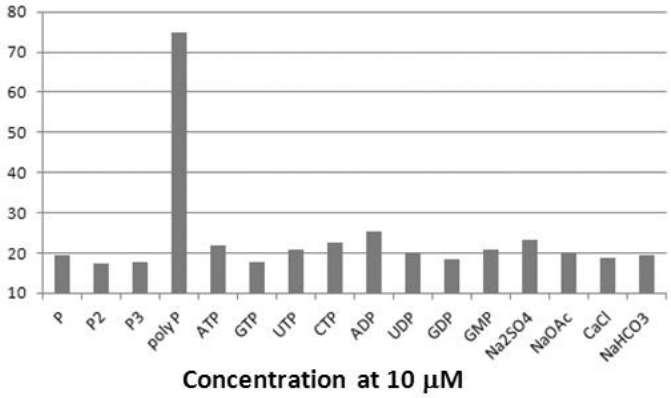
ProFoldin
MicroMolar Polyphosphate Assay Kit

CATALOG NUMBER PPD1000

INTRODUCTION

Inorganic polyphosphate is a linear molecule composed of tens or hundreds of phosphate residues linked together. In bacteria, polyphosphate kinase (PPK) converts polyphosphate and ADP to ATP. Lacking PPK activity resulted in polyphosphate deficiency and failure in expression of RpoS (a sigma factor for RNA polymerase) that leads to cell death. Thus PPK is a potential antibacterial drug target. The MicroMolar Polyphosphate Assay Kit is for measurement of micromolar concentrations of polyphosphate. The assay is based on increase of the fluorescence intensity (emission 550 nm, excitation 415 nm) of the kit fluorescence dye PPD upon binding to polyphosphate. The assay is compatible with regular buffers and various phosphate compounds including inorganic phosphate, pyrophosphate, ATP, ADP and AMP. The assay kit can be used for measurements of polyphosphate in biological samples or environmental water samples.





The kit (catalog number PPD1000) includes 300 µl of 100 x PPD dye and 30 µl of 1 mM sodium polyphosphate (a 45-mer). It is for 1000 assays using 384-well plates (30 µl of sample volume) or 500 samples using 96-well plates (60 µl of sample volume). Cuvettes may also be used for measurements.

Reference:

1. Achbergerová L. et al, Degradation of polyphosphates by polyphosphate kinases from Ruegeria pomeroyi. Biotechnology Letters. Volume 36, Issue 10, pp 2029-2035 (2014).
2. Zhang J. et al, A Fast Sensor For in Vivo Quantification of Cytosolic Phosphate in Saccharomyces Cerevisiae, Biotechnol Bioeng.Vol 112, pp 1033-1046 (2015).

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INSTRUCTIONS

PROTOCOL

Standard curve

1. **Sample preparation:** Prepare the polyphosphate solutions, 45-mer sodium polyphosphate, at a series of concentrations ranging from 50 μM to zero in a 10 mM HEPES, pH 7.4 buffer. Dilute the 100 x PPD dye 100-fold with water to make the 1 x PPD dye.

> Note: The fluorescence intensity has a linear relationship with the polyphosphate concentrations in the range of 1 to 50 μM. A polyphosphate concentration higher than 50 μM suppresses the fluorescence signal.

2. **Detection:** Mix 30 μl of the sample with 30 μl of the 1x PPD dye solution for 5 min. Read the fluorescence intensity at 550 nm (excitation 415 nm).

3. **Data Analysis**: Plot the fluorescence intensity **Fc** and the polyphosphate concentration **[Polyphosphate]** to generate the linear standard curve.

$$\mathbf{Fc = a\,[Polyphosphate] + b}$$

Where the **Fc** values are from experimental data, the **a** and **b** values are from the linear fitting between the **Fc** values and the polyphosphate concentrations.

UNKNOWN SAMPLES

Follow the same procedure to measure the fluorescence intensity **Fc** values from the unknown samples. Calculate the polyphosphate concentrations in the unknown samples using the **Fc** values from the unknown samples and the **a** and **b** values from the standard curve.

$$\mathbf{[Polyphosphate] = (Fc - b) / a}$$

RELATED PRODUCTS

MPA3000	MicroMolar Phosphate Assay Kit
NPA1000	NanoMolar Phosphate Assay Kit
EPA001	Easy Protein Assay Reagent
HIS200	MicroMolar Histidine Assay Kit
CYS200	MicroMolar Cysteine Assay kit
PAA100K	MicroMolar Primary Amine Assay Kit
EDTA200	MicroMolar EDTA Assay kit
DTT200	MicroMolar DTT Assay kit
DAK1000	Detergent assay kit
LIP1000	MicroGram Lipid Assay Kit

For more information of concentration assays and enzyme essays, please visit www.profoldin.com.