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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. Studies also suggested that large accumulation of polyphosphate in cells responses to deficiencies in an amino acid, phosphate or nitrogen.

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) provides the reagent for measurement of 1000 samples 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. The kit also provides a polyphosphate, a 45-mer of sodium polyphosphate, as a control.

PROTOCOL

1. **Sample preparation:** Prepare the polyphosphate solutions 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. The sodium polyphosphate, a 45-mer, provided with the kit can be used as a control.

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) and plot the correlation between the fluorescence signal and the polyphosphate concentration.

> 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. For more information, please see the product web page at http://www.profoldin.com/polyphosphate_assay.html.