



ProFoldin

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INSTRUCTIONS

ProFoldin MicroMolar ADP Assay Kits

MicroMolar ADP assay kit

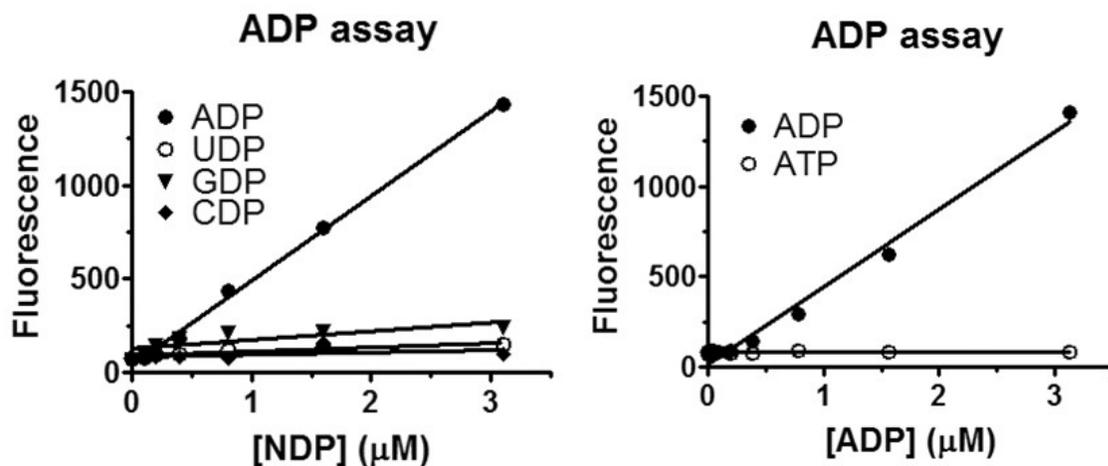
Catalog number: MAD100K

MicroMolar ADP assay kit - 500

Catalog number: MAD500K

INTRODUCTION

The MicroMolar ADP Assay is for measurement of ADP concentrations in a sub-micromolar to low micromolar range. Other nucleoside diphosphates (UDP, GDP and CDP) showed very little background. Nucleoside triphosphates (ATP, UTP, GTP and CTP) and monophosphates (AMP, UMP, GMP and CMP) are not detected. The ADP assay is based on fluorescence measurement with emission at 535 nm and excitation at 485 nm. It is a high throughput assay using regular black 96-well or 384-well plates. Microcuvettes may also be used for detection of the fluorescence signals. The assay can be used to measure contamination of ADP in ATP samples or monitor hydrolysis of ATP. It can also be used for measurement of kinase activities and other enzyme activities that generate ADP molecules. The ADP assay is compatible with common reaction buffers with magnesium. It is not compatible with samples containing high concentrations of DNA or RNA.



MicroMolar ADP assay kit (Catalog number: MAD100K): The kit contains 350 μ l of 10 x Buffer, 30 μ l of 100 x MAD reagent 1, 30 μ l of 100 x MAD reagent 2 and 300 μ l of 10 x fluorescence dye. The assay reagents are sufficient for measurement of 100 samples of ADP using a standard black 384-well plate.

MicroMolar ADP assay kit - 500 (Catalog number: MAD500K): The kit contains 1800 μ l of 10 x Buffer, 150 μ l of 100 x MAD reagent 1, 150 μ l of 100 x MAD reagent 2 and 1500 μ l of 10 x fluorescence dye. The assay reagents are sufficient for measurement of 500 samples of ADP using a standard black 384-well plate.



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PROTOCOL

The following assay protocol is based on using a 384-well plate (Matrix 4318 plate) for the measurement. The sample volume is 15 µl and the final assay volume is 30 µl. For 96-well plate assays, the sample volume is 50 µl and the final assay volume is 100 µl. For assays using cuvette, the sample volume is 500 µl and the final assay volume is 1000 µl.

STANDARD CURVE

1. **Sample preparation:** Prepare 15 µl of ADP solutions in a 384-well black plate (Matrix 4318 plate) with a two-fold serial dilution from 0.005 mM to zero in water. Freshly prepare the following premix for each 10 assays: 136.8 µl of H₂O, 36 µl of 10 x Buffer, 3.6 µl of 100 x MAD Reagent 1, 3.6 µl of 100 x MAD Reagent 2.

The 1 x Buffer is composed of 50 mM Tris-HCl, pH 8.0, 3 mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 0.003% Brij-35.

2. **Detection:** Mix 15 µl of the ADP samples with 15 µl of the premix for 45 min. Dilute 33 µl of the 10 x Fluorescence dye with 297 µl of water (10-fold dilution). Add 30 µl of 1 x Fluorescence dye and read the fluorescence intensity at 535 nm with excitation at 485 nm in 5 min.
3. **Data Analysis:** Plot the fluorescence intensity **F_c** and the ADP concentration [**ADP**] to generate the linear standard curve.

$$F_c = a [\text{ADP}] + b$$

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

UNKNOWN SAMPLES

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

$$[\text{ADP}] = (F_c - b) / a$$

RELATED PRODUCTS

MicroMolar UDP assay kit

Catalog number: MUD100K

MicroMolar UDP assay kit - 500

Catalog number: MUD500K

MicroMolar GDP assay kit

Catalog number: MGD100K

MicroMolar GDP assay kit - 500

Catalog number: MGD500K

MicroMolar Universal Kinase Assay Kit-500 (kinase not included)

Catalog Number: MUK500K

Thymidylate Kinase Assay Kit Plus-500 (kinase included)

Catalog number: TMK500KE

Human Thymidylate Kinase Assay Kit Plus-500 (enzyme included)

Catalog Number: HTMK500KE