



## ProFoldin

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# INSTRUCTIONS

## ProFoldin MicroMolar GDP Assay Kits

**MicroMolar GDP assay kit**

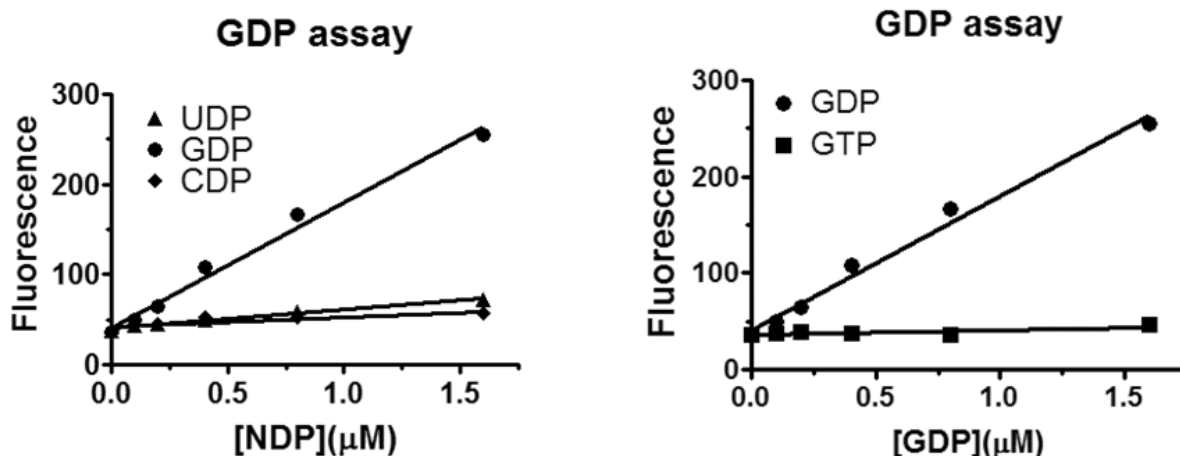
**Catalog number: MGD100K**

**MicroMolar GDP assay kit - 500**

**Catalog number: MGD500K**

### INTRODUCTION

The MicroMolar GDP Assay is for measurement of GDP concentrations in a sub-micromolar to low micromolar range. UDP and CDP showed very little background. ADP will also be detected. Nucleoside triphosphates (ATP, UTP, GTP and CTP) and monophosphates (AMP, UMP, GMP and CMP) are not detected. The GDP 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. Micro-cuvettes may also be used for detection of the fluorescence signals. The assay can be used to measure contamination of GDP in GTP samples or monitor hydrolysis of GTP. It can also be used for measurement of enzyme activities that generate GDP molecules. The GDP assay is compatible with common reaction buffers with magnesium. It is not compatible with samples containing ADP or high concentrations of DNA or RNA.



**MicroMolar GDP assay kit (Catalog number: MGD100K):** The kit contains 350  $\mu$ l of 10 x Buffer, 30  $\mu$ l of 100 x MGD reagent 1, 30  $\mu$ l of 100 x MGD reagent 2, and 300  $\mu$ l of 10 x fluorescence dye. The assay reagents are sufficient for measurement of 100 samples of GDP using a standard black 384-well plate.

**MicroMolar GDP assay kit - 500 (Catalog number: MGD500K):** The kit contains 1800  $\mu$ l of 10 x Buffer, 150  $\mu$ l of 100 x MGD reagent 1, 150  $\mu$ l of 100 x MGD reagent 2 and 1500  $\mu$ l of 10 x fluorescence dye. The assay reagents are sufficient for measurement of 500 samples of GDP 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 GDP 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 H<sub>2</sub>O, 36 µl of 10 x Buffer, 3.6 µl of 100 x MGD Reagent 1, 3.6 µl of 100 x MGD Reagent 2.

The 1 x Buffer is composed of 50 mM Tris-HCl, pH 8.0, 3 mM MgCl<sub>2</sub>, 0.2 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 0.003% Brij-35.

2. **Detection:** Mix 15 µl of the GDP samples with 15 µl of the premix for 45 min. Dilute 33µl 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<sub>c</sub>** and the GDP concentration [**GDP**] to generate the linear standard curve.

$$\mathbf{F_c} = \mathbf{a} [\mathbf{GDP}] + \mathbf{b}$$

Where the **F<sub>c</sub>** values are from experimental data, the **a** and **b** values are from the linear fitting between the **F<sub>c</sub>** values and the GDP concentrations.

## UNKNOWN SAMPLES

Follow the same procedure to measure the fluorescence intensity **F<sub>c</sub>** values from the unknown samples. Calculate the GDP concentrations in the unknown samples using the **F<sub>c</sub>** values from the unknown samples and the **a** and **b** values from the standard curve.

$$[\mathbf{GDP}] = (\mathbf{F_c} - \mathbf{b}) / \mathbf{a}$$

## RELATED PRODUCTS

MicroMolar ADP assay kit

Catalog number: MAD100K

MicroMolar UDP assay kit

Catalog number: MUD100K

For more concentration assays of various biochemical molecules and inorganic ions, please visit our website at [www.profoldin.com](http://www.profoldin.com).