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INSTRUCTIONS

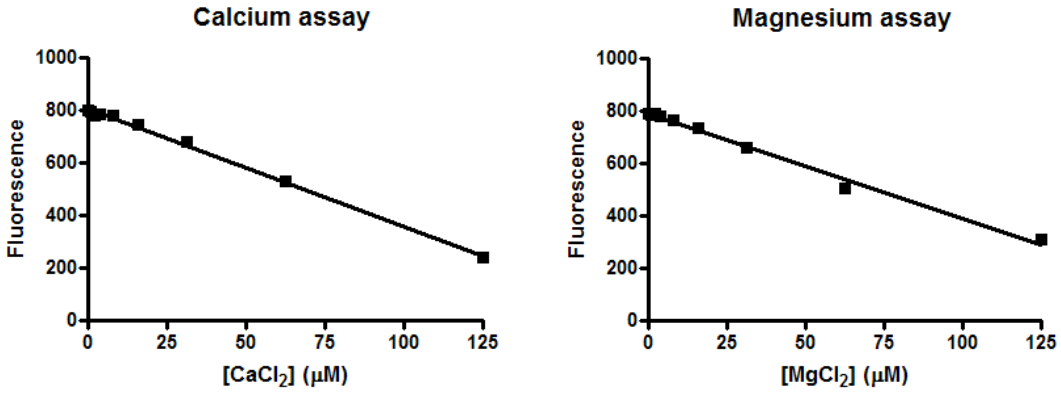
ProFoldin
MicroMolar Calcium / Magnesium Assay Kit

CATALOG NUMBER **DMA200**

INTRODUCTION

Calcium (Ca^{2+}) or Magnesium (Mg^{2+}) is an essential metal ion in biological systems. These divalent ions are also common components in biochemical buffers and pharmaceutical products. The MicoMolar Calcium / Magnesium Assay Kit is for measurement of micromolar concentrations of calcium, and magnesium (10 µM – 120 µM). The assay may also be used to detect other divalent metal ions. The assay is based on the complex formation of the divalent metal ion with the assay reagent that results in reduction of the fluorescence intensity (emission 535 nm, excitation 485 nm). Chelators such EDTA and thiol compounds bind divalent ions and should be avoided in the assay. The assay is compatible with HEPES buffer, low concentrations of non-ionic detergent (<0.01%), Tris-HCl (<10 mM), and phosphate (< 1 mM). It is not compatible with thiol compounds such as DTT, 2-mercaptoethanol or cysteine. It is interfered by divalent metal ions such as Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Zn^{2+}. For specific assays of transitional metal ions such as Cu^{2+} and Zn^{2+}, please visit http://www.profoldin.com/concentration.html.

The assay kit can be used for measurements calcium or magnesium concentrations in buffer samples or pharmaceutical products.



The MicroMolar Calcium / Magnesium Assay Kit (catalog number DMA200) includes 200 µl of 100 x C56 dye and 1000 µl of 10 x Reagent E and 50 µl of $CaCl_2$. It is for measurement of 200 samples using 96-well plates. Cuvettes may also be used for measurements.

ASSAY PROTOCOL

The following assay protocol is based on using a 96-well plate for the measurement. The sample volume is 100 µl and the final assay volume is 250 µl. For assays using cuvette, the sample volume is 400 µl and the final assay volume is 1000 µl.

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STANDARD CURVE

1. **Sample preparation:** Prepare 100 µl of $CaCl_2$ solutions in the wells of a black 96-well plate with a two-fold serial dilution from 0.2 mM to zero in water or a 10 mM HEPES, pH 7.4 buffer. For 10 samples, dilute 0.011 ml of the 100 x C56 dye 100-fold with water to make 1.1 ml of 1 x C56 dye and dilute 0.055 ml of 10 x Reagent E with water to make 0.55 ml of 1 x Reagent E.

2. **Detection:** Mix 50 µl of 1 x Reagent E with 100 µl of the $CaCl_2$ solutions for 2 min. Then add 100 µl of 1 x C56 dye and incubate the solution in the dark for 15 min. Finally read the fluorescence at 535 nm (excitation at 485 nm).

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

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

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

UNKNOWN SAMPLES

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

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

RELATED PRODUCTS

MCA1000	MicroMolar Copper Assay Kit
NZA1000	NanoMolar Zinc Assay Kit
MPA3000	MicroMolar Phosphate Assay Kit
NPA1000	NanoMolar Phosphate Assay Kit
PPD1000	MicroMolar Polyphosphate Assay Kit
MSA200	MicroMolar Sulfate Assay Kit
CLA100	MicroMolar Chloride Assay Kit
HIS200	MicroMolar Histidine Assay Kit
CYS200	MicroMolar Cysteine Assay kit
EDTA200	MicroMolar EDTA Assay kit
DTT200	MicroMolar DTT Assay kit
DAK1000	Detergent assay kit
SDS200	NanoGram SDS Assay Kit
CMC1000	Detergent Critical Micelle Concentration (CMC) Assay Kit
LIP1000	MicroGram Lipid Assay Kit
MAD100K	MicroMolar ADP Assay kit