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INSTRUCTIONS

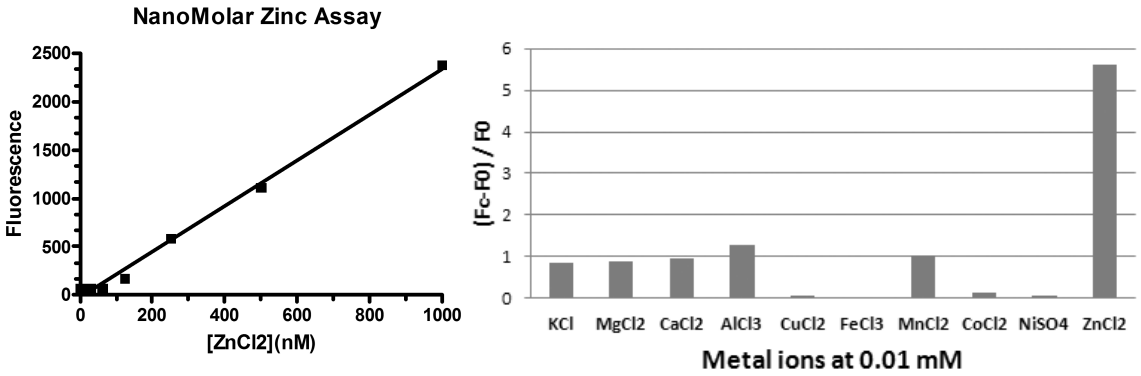
ProFoldin
NanoMolar Zinc Assay Kit

CATALOG NUMBER **NZA1000**

INTRODUCTION

Zinc (Zn^{++}) is an essential metal ion in biological systems. Zinc is a cofactor of hundreds of enzymes and plays important roles in signal transduction, gene expression, regulation of apoptosis, synaptic plasticity and prostate gland function. Zinc deficiency is associated with malabsorption syndrome, chronic GI, liver disease, diabetes, renal disease, sickle cell disease, anorexia nervosa, and HIV infection.

The NanoMolar Zinc Assay Kit is for measurement of submicromolar concentrations of zinc (0.1 µM – 2 µM). The assay is based on the principle that binding the fluorescence dye NZA selectively with Zinc results in increase of the fluorescence intensity (emission 535 nm, excitation 485 nm). The assay is compatible with regular buffers with different metal ions including 10 µM Mg^{2+}, Ca^{2+}, Cu^{2+}, Mn^{2+}, Al^{3+}, Fe^{3+}, Ag^{+}, Co^{2+} and Ni^{2+}. Chelators such EDTA and thiol compounds bind zinc and should be avoided in the assay. The assay kit can be used for high-throughput measurements of zinc concentrations in biochemical assay reactions associated with zinc metabolism or environmental water samples.



The NanoMolar Zinc Assay Kit (catalog number NZA1000) includes 500 µl of 100 x NZA dye and 25 µl of 1 mM $ZnCl_2$. It is for 1000 assays using 96-well plates. Cuvettes may also be used for measurements.

PUBLICATION

Dandley E. C. et al, Atomic layer deposition coating of carbon nanotubes with zinc oxide causes acute phase immune responses in human monocytes in vitro and in mice after pulmonary exposure. Particle and Fibre Toxicology, Vol.13: 29 (2016).

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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 150 µl. For 384-well plate assays, the sample volume is 60 µl and the final assay volume is 90 µl. For assays using cuvette, the sample volume is 800 µl and the final assay volume is 1200 µl.

STANDARD CURVE

1. **Sample preparation:** Prepare 100 µl of $ZnCl_2$ solutions in the wells of a black 96-well plate with a two-fold serial dilution from 0.005 mM to zero in a 10 mM Tris-HCl or HEPES buffer, pH 7.4. Dilute the 100 x NZA dye 100-fold with water to make the 1 x NZA dye.

2. **Detection:** Mix 50 µl of 1 x NZA dye with 100 µl of the $ZnCl_2$ solutions for 5 min and read the fluorescence at 535 nm (excitation at 485 nm).

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

$$\mathbf{Fc = a\,[Zn] + Fo}$$

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

UNKNOWN SAMPLES

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

$$\mathbf{[Zn] = (Fc - Fo) / a}$$

RELATED PRODUCTS

MCA1000	MicroMolar Copper Assay Kit
NMA1000	NanoMolar Nickel / Cobalt Assay Kit
DMA200	MicroMolar Calcium / Magnesium Assay Kit
NPA1000	NanoMolar Phosphate Assay Kit
PPD1000	MicroMolar Polyphosphate Assay Kit
MSA200	MicroMolar Sulfate Assay Kit
EDTA200	MicroMolar EDTA Assay kit
DAK1000	Detergent assay kit
LIP1000	MicroGram Lipid Assay Kit
MUD100K	MicroMolar UDP assay kit
PAA100K	MicroMolar Primary Amine Assay Kit

For more concentration assays, please visit our website at www.profoldin.com.