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INSTRUCTIONS

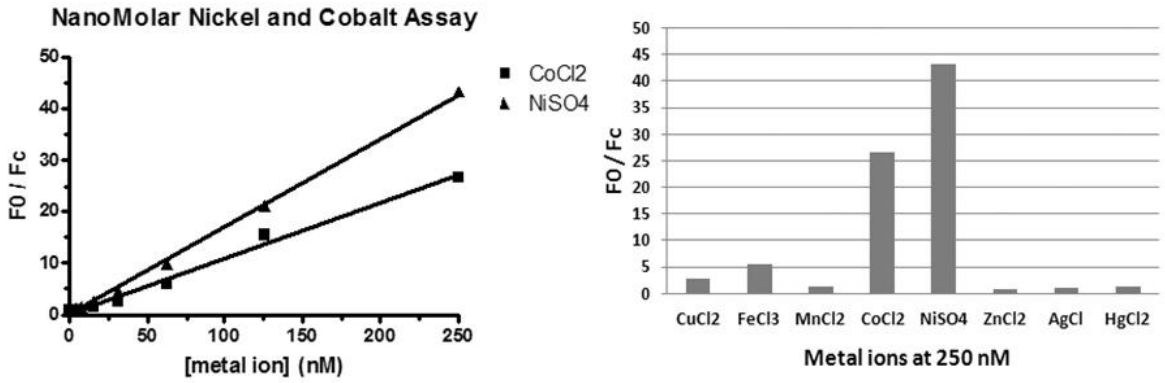
ProFoldin
NanoMolar Nickel / Cobalt Assay Kit

CATALOG NUMBER **NMA1000**

INTRODUCTION

Nickel (Ni^{++}) and cobalt (Co^{++}) are essential metal ions in biological systems. Many enzymes such as methionine aminopeptidase and glucose isomerase contain cobalt. Some other enzymes such as ureases from bactaria and plants use nickel as a cofactor. Synthesis of Ni / Co enzymes and coenzyme B12 requires high-affinity uptake of the metal ions from natural environments. In bacteria, Ni and Co uptake is mediated by secondary transporters and ATP-binding cassette systems. Understanding the differences between cobalt and nickel transporters might lead to drug development for gastritis and peptic ulceration.

The NanoMolar Nickel / Cobalt Assay Kit is for measurement of nanomolar concentrations of nickel or cobalt. The assay is based on the principle that binding the fluorescence dye NMA with nickel or cobalt ions results in decrease of the fluorescence intensity (emission 535 nm, excitation 485 nm). Other metal ions including Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Al^{3+}, Cu^{2+} and Ag^{+} give much lower assay sensitivity. Chelators such EDTA and thiol compounds bind strongly some of the metal ions and should be avoided in the assay.



F_0 is the fluorescence when the Ni of Co concentration is zero. F_c is the fluorescence when the Ni of Co concentration is [Ni] or [Co].

The assay kit can be used for high-throughput measurements of nickel or cobalt concentrations in biological samples or environmental water samples. The kit can also be used for biochemical assays of enzyme assays associated with nickel or cobalt metabolism.

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The kit includes 300 µl of 100 x NMA dye. It is for 1000 assays using 384-well plates (30 µl of sample volume) or 500 assays using 96-well plates (60 µl of sample volume). Cuvettes may also be used for measurements.

PROTOCOL

Standard curve

1. **Sample preparation:** Prepare the metal (nickel sulfate or cobalt chloride) solutions at a series of concentrations ranging from 1 µM to zero in 10 mM HEPES, pH 7.5. Dilute the 100 x NMA dye 100-fold with water to make the 1 x NMA dye.

2. **Detection:** Mix 30 µl of the sample with 30 µl of the 1x NMA dye solution for 10 min. Read the fluorescence intensity (Fc) at 535 nm (excitation 485 nm). The fluorescence for the buffer or water without metal is Fo.

3. **Data Analysis**: Calculate the Fo / Fc values and plot the correlation between the Fo / Fc values and the metal ion concentrations **[M]** (Ni^{++} or Co^{++}).

$$\mathbf{Fo / Fc = a [M] + b}$$

Where the **Fo / Fc** and **[M]** values are from experimental data, the **a** and **b** values are from the linear fitting between the **Fo / Fc** values and the metal concentrations.

UNKNOWN SAMPLES

Follow the same procedure to measure the fluorescence intensity **Fc** values from the unknown samples and calculate the **Fo / Fc** values. Calculate the metal ion concentrations **[M]** in the unknown samples using the **Fo / Fc** values from the unknown samples and the **a** and **b** values from the standard curve.

$$\mathbf{[M] = (Fo / Fc – b) / a}$$

RELATED PRODUCTS

NZA1000	NanoMolar Zinc Assay Kit
MCA1000	MicroMolar Copper Assay Kit
NPA1000	NanoMolar Phosphate Assay Kit
PPD1000	MicroMolar Polyphosphate Assay Kit
CYS200	MicroMolar Cysteine Assay kit
EDTA200	MicroMolar EDTA Assay kit
DTT200	MicroMolar DTT Assay kit
DAK1000	Detergent assay kit
SCMC1000	Detergent Critical Micelle Concentration (CMC) Assay Kit
LIP1000	MicroGram Lipid Assay Kit
MSA200	MicroMolar Sulfate Assay Kit

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