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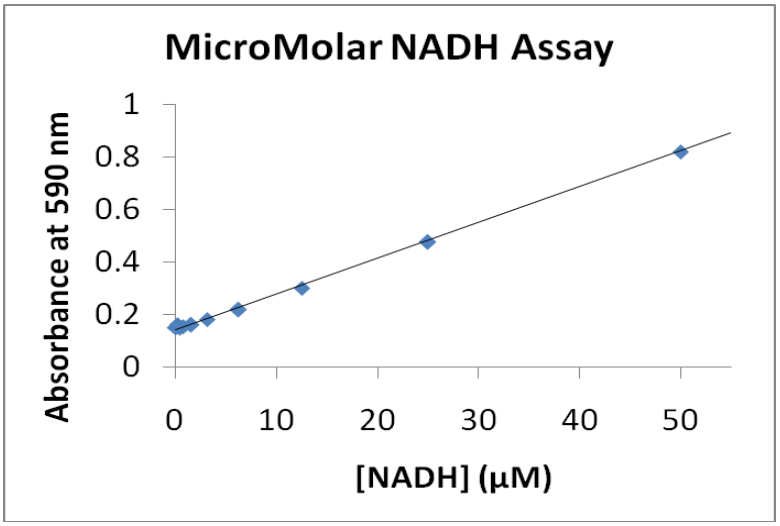
INSTRUCTIONS

ProFoldin
MicroMolar NADH Assay Kit

CATALOG NUMBER **NADH100**

INTRODUCTION

The assay measures micromolar concentrations of NADH. NADH is the reduced form of nicotinamide adenine dinucleotide (NAD). NADH is an enzymatic cofactor for many reductases and acts as an electron donor. It is a product of many oxidases using NAD as an electron acceptor. Both NADH and NAD are involved in many biosynthesis and degradation pathways. The MicroMolar NADH Assay Kit (NADH100) provides a convenient tool for sensitive detection of NADH. The assay is based on detection of light absorbance at 590 nm and the assay process is completed within 20 min. It is in a 96-well plate format and can be used for high throughput screening of enzymes involving NADH. The assay is compatible with most common buffers in biochemistry labs. It is not compatible with NADPH or other reducing reagents such DTT.



The **MicroMolar NADH Assay Kit (Catalog No. NADH100)** includes 1 ml of 10x Assay buffer, 0.1 ml of 20 x Reagent A, 0.1 ml of 20 x Reagent B, 0.5 ml of 10x Reagent C and 0.020 ml of 10 mM NADH. It is for 100 assays in a 96-well plate format.

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 190 µl. It can be reformatted in a 384-well plate assays or using a cuvette by adjusting the volumes accordingly.

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STANDARD CURVE

1. **Reagent dilution and sample preparation:** Dilute the 20 x stock solutions 20-fold with water. Dilute the 10 x stock solutions 10-fold with water. Prepare 100 µl of NADH solutions in the wells of a clear 96-well plate with a two-fold serial dilution from 100 µM to zero in 1 x assay buffer.

2. **Detection:** Into the 100 µl of the NADH solutions, add 20 µl of 1 x Reagent A, then 20 µl of 1 x Reagent B. Incubate the mixture at room temperature for 10 min then add 50 µl of 1 x Reagent C and read the light absorbance at 590 nm.

3. **Data Analysis**: Plot the light absorbance **Ac** and the NADH concentration **[NADH]** to generate the linear standard curve.

$$\mathbf{Ac = a\ [NADH] + b}$$

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

UNKNOWN SAMPLES

Follow the same procedure to measure the light absorbance **Ac** values from the unknown samples. Calculate the NADH concentrations in the unknown samples using the **Ac** values from the unknown samples and the **a** and **b** values from the standard curve.

$$\mathbf{[NADH] = (Ac - b) / a}$$

RELATED PRODUCTS

NADPH100	MicroMolar NADPH Assay Kit
PPD1000	MicroMolar Polyphosphate Assay Kit
HIS200	MicroMolar Histidine Assay Kit
CYS200	MicroMolar Cysteine Assay kit
PEP200	Peptide Assay Kit
PAA100K	MicroMolar Primary Amine Assay Kit
CAK1000	Coenzyme A Assay Kit
EDTA200	MicroMolar EDTA Assay kit
DAK1000	Detergent Assay Kit
CMC1000	Detergent Critical Micelle Concentration (CMC) Assay Kit
LIP1000	MicroGram Lipid Assay Kit
NPA1000	NanoMolar Phosphate Assay Kit
MAD100K	MicroMolar ADP Assay kit
MUD100K	MicroMolar UDP assay kit
MCA1000	MicroMolar Copper Assay Kit
NZA1000	NanoMolar Zinc Assay Kit
NMA1000	NanoMolar Nickel / Cobalt Assay Kit
MSA200	MicroMolar Sulfate Assay Kit

For more information of concentration assays, please visit www.profoldin.com.