Cell Meter™ Intracellular NADH/NADPH Flow Cytometric Analysis Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 15291 (100 assays)	Keep in freezer, avoid light	Flow Cytometer

Introduction

The detection of intracellular dihydronicotinamide adenine dinucleotide NADH and its phosphate ester NADPH is important for disease diagnostics and drug discovery. In general, the redox couples NAD/NADH and NADP/NADPH play a critical role in energy metabolism, glycolysis, tricarboxylic acid cycle and mitochondrial respiration. The increased NAD(P)H level in cells is linked to the abnormal production of reactive oxygen species (ROS) and DNA damage. However, due to the lack of sensitive NAD(P)H probe, it has been challenging to detect intracellular NAD(P)H in biological systems. Cell Meter™ Intracellular NADH/NADPH Flow Cytometric Analysis Kit provides an efficient method to monitor intracellular NAD(P)H level in live cells.

JZL1707 NAD(P)H sensor has been developed as an excellent fluorescent probe for detecting and imaging NADH/NADPH in cells. The probe bind NADH/NADPH to generate strong fluorescence signal with high sensitivity and specificity. JZL1707 NAD(P)H sensor can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using flow cytometer in PE channel.

Kit Components

Components	**Amount**
Component A: JZL1707 NAD(P)H sensor	100 µL
Component B: Assay Buffer	1 bottle (50 mL)

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells (0.5 -1×10^6 cells/mL) → Incubate cells with test compounds and JZL1707 NAD(P)H Sensor at 37 ºC for 30-60 minutes → Wash and keep cells in Assay Buffer → Analyze cells with a flow cytometer

Note: Thaw all the components at room temperature before use.

1. For each sample, prepare cells in 0.5 mL serum-free medium or buffer of your choice at a density of 1×10^5 to 1×10^6 cells/mL.
   Note 1: Each cell line should be evaluated on an individual basis to determine the optimal cell density. For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-free media.
   Note 2: JZL1707 NAD(P)H sensor is serum sensitive, therefore it's recommended to keep cells in serum-free medium or buffer of your choice. Alternatively, cells can be prepared and treated in regular full medium. Change to serum-free medium or buffer of your choice when incubation with JZL1707 NAD(P)H sensor.

2. Incubate cells with test compounds at 37 ºC for a desired period of time to stimulate intracellular NADH/NADPH.
   Note: The appropriate incubation time depends on the individual cell type and test compound used. Optimize the incubation time for each experiment.

3. Add 1 µL of JZL1707 NAD(P)H sensor (Component A) into 0.5 mL cell suspension. Incubate at 37 ºC for 30-60 minutes.
   Note: For a NADH/NADPH positive control treatment: Jurkat cells were incubated with 100 µM NADH or NADPH for 30 minutes in serum-free medium, and co-incubated with JZL1707 NAD(P)H sensor working solution at 37 ºC for another 30 minutes. See Figure 1 for details.

4. Wash cells with your desired buffer once. Keep cells in Assay Buffer (Component B).

5. Monitor the fluorescence intensity at PE channel using a flow cytometer. Gate on the cells of interest, excluding debris.

Data Analysis

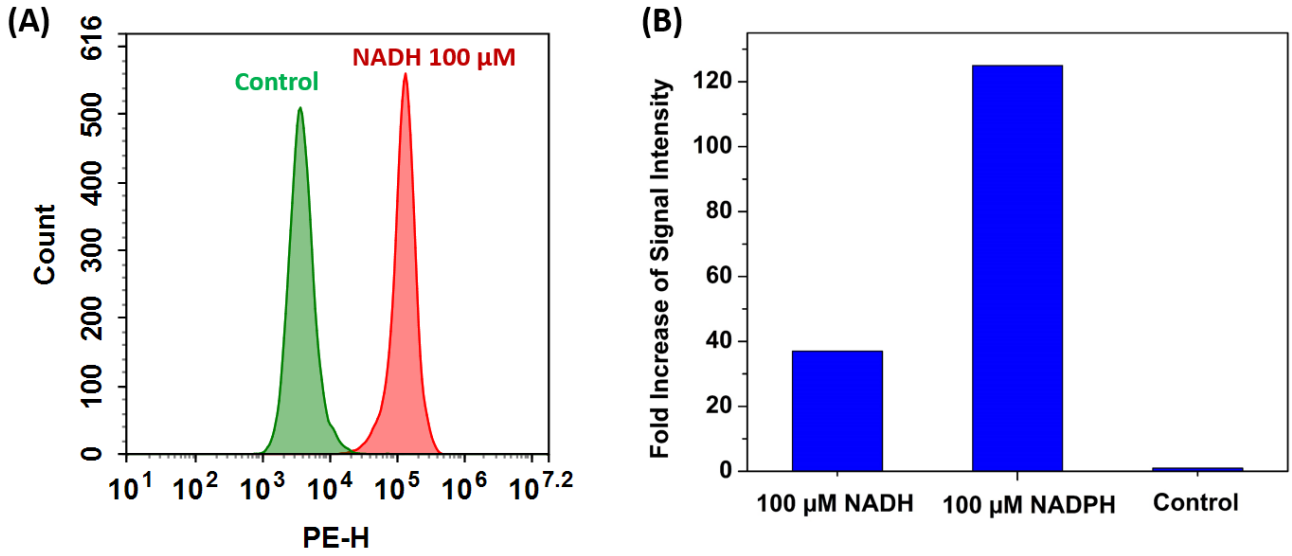

Figure 1. (A) Flow cytometric analysis of NADH/NADPH measurement in Jurkat cells using Cell Meter™ Intracellular NADH/NADPH Flow Cytometric Analysis Kit (Cat#15291). Cells were incubated with or without 100 µM NADH in serum-free medium for 30 minutes and then co-incubated with JZL1707 NAD(P)H sensor working solution for another 30 minutes. (B) Fold increase of fluorescence signal intensity of Jurkat cells treated with 100 µM NADH or 100 µM NADPH compared with untreated control. Fluorescence intensity was measured using ACEA NovoCyte flow cytometer in PE channel.

References

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4. Stephen Y. Xue, Valeria Y. Hebert, Danicia M. Hayes, Corie N. Robinson, Mitzi Glover, and Tammy R. Dugas. Toxicol. Sci., Aug 2013; 134: 323 - 334