

Cell Meter™ Intracellular NADH/NADPH Flow Cytometric Analysis Kit

Catalog number: 15291
Unit size: 100 Tests

Component	Storage	Amount
Component A: JZL1707 NAD(P)H sensor	Freeze (<-15 °C), Minimize light exposure	100 uL
Component B: Assay Buffer	Freeze (<-15 °C)	50 mL

OVERVIEW

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 binds 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.

AT A GLANCE

Protocol summary

1. Prepare cells (0.5 - 1×10⁶ cells/mL)
2. Incubate cells with test compounds and JZL1707 NAD(P)H Sensor at 37 °C for 30-60 minutes
3. Wash and keep cells in Assay Buffer
4. Analyze cells with a flow cytometer using PE channel

Important

Thaw all the kits components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	575/26 nm filter
Instrument specification(s):	PE channel

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit <https://www.aatbio.com/resources/guides/cell-sample-preparation.html>

SAMPLE EXPERIMENTAL PROTOCOL

1. For each sample, prepare cells in 0.5 mL serum-free medium or buffer of your choice at a density of 1×10⁵ to 1×10⁶ cells/mL.

Note 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 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 °C 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 °C 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 °C 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.

EXAMPLE DATA ANALYSIS AND FIGURES

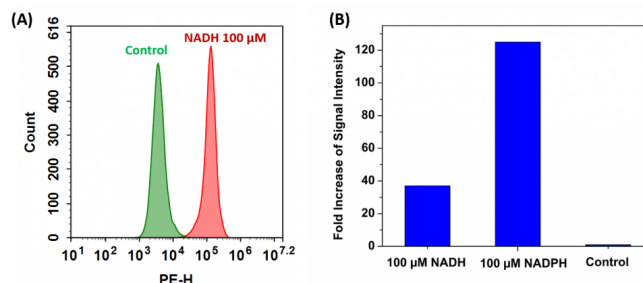


Figure 1.

(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.

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