Cell Meter™ Intracellular NADH/NADPH Flow Cytometric Analysis Kit *Deep Red Fluorescence*

Catalog number: 15296
Unit size: 100 Tests

Component	Storage	Amount
Component A: JJ1902 NAD(P)H Sensor	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (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 in the far spectrum and can be combined with other applications such as GFP-expressed cells or application of MitoTracker. JJ1902 NAD(P)H sensor has been developed as an excellent fluorescent probe for detecting and imaging NADH/NADPH in cells. The probe which is fluorogenic in nature, binds NADH/NADPH to generate strong fluorescence signal with high sensitivity and specificity. JJ1902 NAD(P)H sensor can be readily loaded into live cells, and its fluorescence signal can be conveniently monitored using flow cytometer in APC channel.

AT A GLANCE

Protocol summary

1. Prepare cells (0.5 - 1×10^6 cells/mL)
2. Incubate cells with test compounds and JJ1902 NAD(P)H Sensor at 37 °C for 20-30 minutes
3. Wash and keep cells in Assay Buffer
4. Analyze cells with a flow cytometer using APC channel

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	640 nm laser
Emission:	660/20 nm filter
Instrument specification(s):	APC 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 1×10^5 to 1×10^6 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 JJ1902 NAD(P)H sensor is compatible in the presence of serum as well. The optimization of the conditions for the sensor is highly recommended cell line to cell line.

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 JJ1902 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 JJ1902 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 such as HHBS or DPBS once. Keep cells in Assay Buffer (Component B).

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

EXAMPLE DATA ANALYSIS AND FIGURES

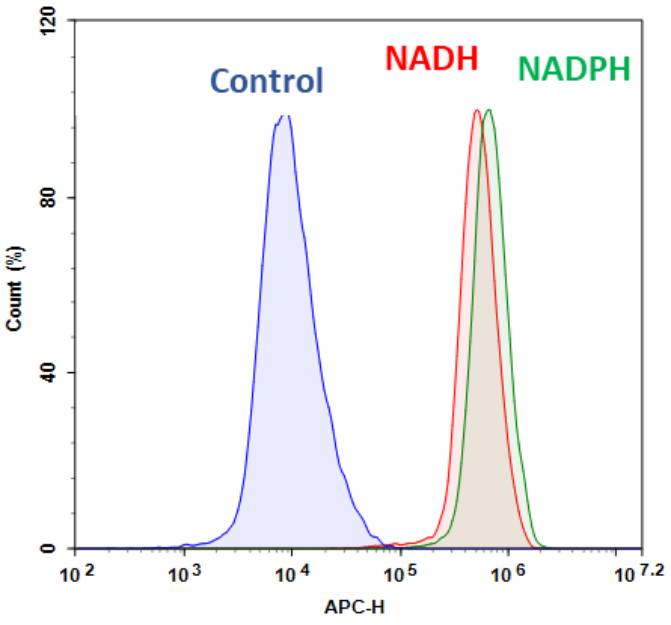

Figure 1. Flow cytometric analysis of NADH/NADPH measurement in Jurkat cells using Cell Meter™ Intracellular NADH/NADPH Flow Cytometric Analysis Kit (Cat#15296). Cells were incubated with or without 100 µM NADH in serum-free medium for 30 minutes and then co-incubated with JJ1902 NAD(P)H sensor working solution for another 30 minutes. Fluorescence intensity was measured

using ACEA NovoCyte flow cytometer in APC channel.

DISCLAIMER