

## Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging Kit \*Deep Red Fluorescence\*

Catalog number: 15295  
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 15295)
Component A: JJ1902 NAD(P)H Sensor	Freeze (< -15 °C), Minimize light exposure	1 vial (40 µL)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 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 Fluorescence Imaging 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 the filter set of Cy5®. This kit is optimized for fluorescence imaging and microplate reader applications.

### AT A GLANCE

#### Protocol Summary

1. Prepare cells in growth medium
2. Incubate cells with test compounds and JJ1902 NAD(P)H Sensor working solution at 37 °C 20 - 30 minutes
3. Wash and keep cells in Assay Buffer
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 590/655 nm (Cutoff = 610 nm) or fluorescence microscope with Cy5® filter

#### Important Note

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

### KEY PARAMETERS

#### Fluorescence microscope

Emission	655 nm
Excitation	590 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Cy5 filter

#### Fluorescence microplate reader

Cutoff	610 nm
Emission	655 nm
Excitation	590 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

### CELL PREPARATION

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

### PREPARATION OF WORKING SOLUTION

Add 10 µL of JJ1902 NAD(P)H Sensor stock solution (Component A) into 2.5 mL of Assay Buffer (Component B), and mix well. This JJ1902 NAD(P)H Sensor working solution is stable within 1 hour at room temperature.

**Note:** 40 µL of JJ1902 NAD(P)H Sensor stock solution is enough for one plate. Protect from light.

### SAMPLE EXPERIMENTAL PROTOCOL

1. To stimulate NADP/NADPH, treat cells with 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X test compounds (384-well plate) in serum free medium or your desired buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of medium or compound buffer.

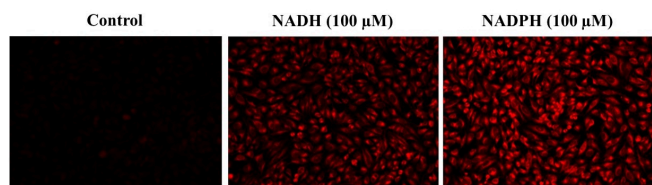
**Note:** JJ1902 NAD(P)H sensor is compatible in the presence of serum as well. The optimization of the conditions for the sensor is highly recommended cell line to cell line.

2. Add 100 µL/well (96-well plate) or 25µL/well (384-well plate) of JJ1902 NAD(P)H Sensor working solution in the cell plate. Co-incubate cells with test compound and JJ1902 NAD(P)H Sensor working solution at 37 °C for 20-30 minutes, protected from light.

**Note:** For a NADH/NADPH positive control treatment: HeLa 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.

3. Wash cells with your desired buffer once. Remove solution in each well and add Assay Buffer (Component B) 100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate.
4. Monitor the fluorescence increase using microplate reader at Ex/Em = 590/655 nm (Cutoff = 610 nm) with bottom read mode, OR take images using fluorescence microscope with the filter set of Cy5® filter.

**EXAMPLE DATA ANALYSIS AND FIGURES**



**Figure 1.** Fluorescence images of NADH/NADPH in HeLa cells using Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging Kit (Cat#15295). HeLa cells were incubated with 100  $\mu$ M NADH or 100  $\mu$ M NADPH in serum-free medium for 30 minutes and then co-incubated with JJ1902 NAD(P)H sensor working solution for another 30 minutes. The fluorescence signal was measured using fluorescence microscope with a Cy5® filter.

**DISCLAIMER**

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