Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 15290 (100 assays)	Keep in freezer, protect from light	Microplate Reader Fluorescence Microscope

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 Fluorescence Imaging 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 the filter set of Cy3® or TRITC. This kit is optimized for fluorescence imaging and microplate reader applications.

Kit Components

Components	**Amount**
Component A: JZL1707 NAD(P)H Sensor	40 µL
Component B: Assay Buffer	1 bottle (20 mL)

Assay Protocol for Plate Reader

Brief Summary

Prepare cells in growth medium → Incubate cells with test compounds and JZL1707 NAD(P)H sensor working solution → Wash and keep cells in Assay Buffer → Monitor fluorescence intensity at Ex/Em = 540/590 nm

1. Prepare cells:

1.1. For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/90 µL for a 96-well plate or 2,500 to 10,000 cells/well/20 µL for a 384-well plate.

1.2. For non-adherent cells: Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 100,000-200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 25,000-50,000 cells/well/20 µL for a 384- well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to your experiment.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare working solution:

2.1 Thaw all the kit component at room temperature before use.

2.2 Make JZL1707 NAD(P)H Sensor working solution: Add 10 µL of JZL1707 NAD(P)H sensor stock solution (Component A) into 2.5 mL of Assay Buffer (Component B), and mix well. The working solution is stable within 1 hour at room temperature.
Note: 40 µL of JZL1707 NAD(P)H sensor stock solution is enough for one plate. Aliquoted and stored unused JZL1707 NAD(P)H sensor stock solution at ≤ -20 °C. Protect it from light and avoid repeated freeze-thaw cycles.

3. Run the NADH/NADPH assay:

3.1 To stimulate NADP/NADPH, treat cells with10 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: JZL1707 NAD(P)H sensor is serum sensitive, therefore it's recommended to keep cells in serum-free medium or the 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.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
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3.2 Add 100 µL/well (96-well plate) or 25µL/well (384-well plate) of JZL1707 NAD(P)H sensor working solution (from Step 2.2) in the cell plate. Co-incubate cells with test compound and JZL1707 NAD(P)H sensor working solution at 37 °C for 30-60 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 JZL1707 NAD(P)H sensor working solution at 37 °C for another 30 minutes. See Figure 1 for details.

3.3 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.

3.4 Monitor the fluorescence increase using microplate reader at Ex/Em = 540/590 nm (cut off = 570 nm) with bottom read mode, or take images using fluorescence microscope with a Cy3® filter.

Data Analysis

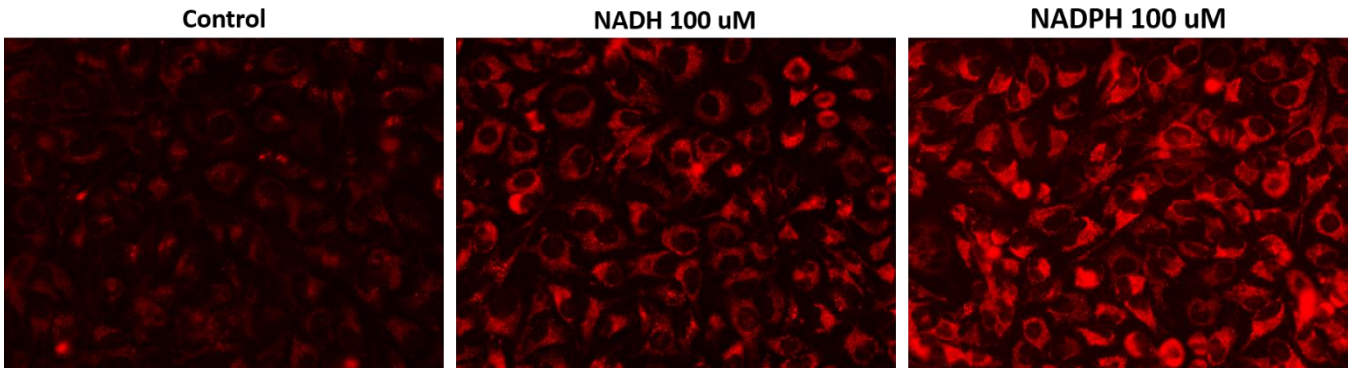

Figure 1. Fluorescence images of NADH/NADPH in HeLa cells using Cell Meter™ Intracellular NADH/NADPH Fluorescence Imaging Kit (Cat#15290). HeLa cells were incubated with 100 µM NADH or 100 µM NADPH in serum-free medium for 30 minutes and then co-incubated with JZL1707 NAD(P)H sensor working solution for another 30 minutes. The fluorescence signal was measured using fluorescence microscope with a Cy3® filter.

References

1. Eugenia Villa-Cuesta, Marissa A. Holmbeckand David M. Rand. Journal of Cell Science (2014) 127, 2282–2290 doi:10.1242/jcs.142026.
2. Chao Tong, Alex Morrison, Samantha Mattison, Su Qian, Mark Bryniarski, Bethany Rankin, Jun Wang, D. Paul Thomas, and Ji Li. FASEB J, Nov 2013; 27: 4332 - 4342.
3. Rubin Tan, Jiansha Li, Xiaochun Peng, Liping Zhu, Lei Cai, Tao Wang, Yuan Su, Kaikobad Irani, and Qinghua Hu. Cardiovasc Res, Oct 2013; 100: 19 - 27.
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