FT-ZE8120

Fluorimetric NAD/NADH ratio Assay Kit

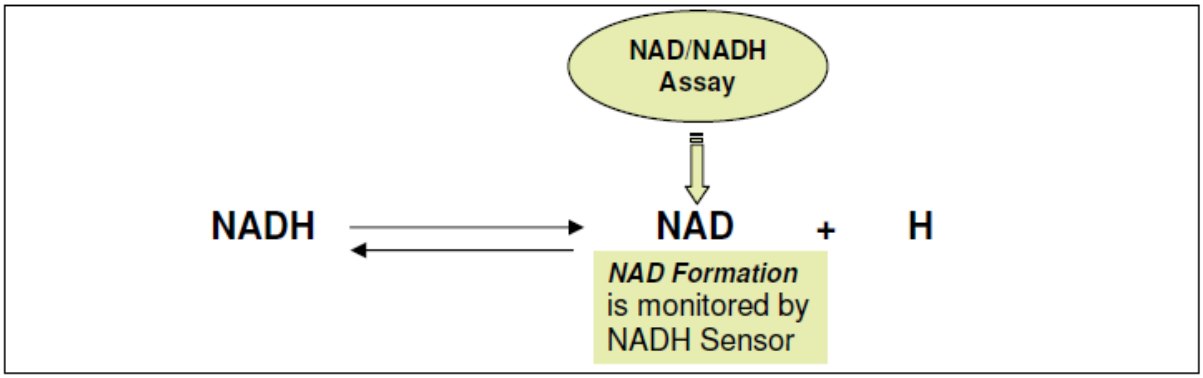
Product Description

Name :	**Fluorimetric NAD/NADH ratio Assay Kit, red fluorescence**	
Catalog Number :	FP-BZ8120	250 assays
Components :	Component A: NAD/NADH Recycling Enzyme Mixture	2 bottles (lyophilized powder)
	Component B: NADH Sensor Buffer	1 bottle (20 mL)
	Component C: NADH Standard	1 vial (142 µg)
	Component D: NADH Extraction Solution	1 bottle (10 mL)
	Component E: NAD Extraction Solution	1 bottle (10 mL)
	Component F: NAD/NADH Control Solution	1 bottle (10 mL)
	Component G: NAD/NADH Lysis Buffer	1 bottle (10 mL)

Storage: -20°C Protect from light and moisture

Introduction

Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are two important cofactors found in cells. NADH is the reduced form of NAD+, and NAD+ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are done by monitoring of NADH or NADPH absorption at 340 nm. This method suffers low sensitivity and high interference since the assay is done in the UV range that requires expensive quartz microplate.



ThisNAD/NADH Ratio Assay Kit provides a convenient method for sensitive detection of NAD, NADH and their ratio. The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction. There is no need to purify NAD/NADH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by either fluorescence microplate reader with Ex/Em = 530 to 570/590 to 600 nm (maximum Ex/Em = 540/590 nm) or absorbance microplate reader at 576±5 nm. This kit also provides cell lysis buffer for convenience.

Kit Key Features

Broad Application:	Can be used for quantifying NAD/NADH in solutions, and in cell extracts.
Sensitive:	The kit detects as low as 10 picomoles of NAD/NADH in solution.
Continuous:	Easily be adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Directions for use

Assay Protocol 'for one 96-well plate) :

Brief Summary

Prepare NADH standards or test samples (25 µL) → Add 25 µL of NADH or NAD Extraction Solution → Incubate at room temperature for 15 min → Add 25 µL of NAD or NADH Extraction Solution (25 µL) → Add NAD/NADH reaction mixture (75 µL) → Incubate at RT for 15 min – 2 hr → Read fluorescence at Ex/Em = 540/590 nm

Note: Thaw1 vial (or bottle) each of all the kit components to room temperature before starting the experiment.

1. Prepare NADH stock solution:

1.1 Prepare NADH standard stock solution: Add 200 µL of PBS buffer into the NADH standard vial (Component C) to have 1 mM (1 nmol/µL) stock solution.
Note: The unused NADH solution should be divided as single use aliquots and stored at -20 °C.

2. Prepare NAD/NADH reaction mixture:

2.1 Prepare the NAD/NADH reaction mixture: Add 10 mL of NADH Sensor Buffer (Component B) to the bottle of NAD/NADH Recycling Enzyme Mixture (Component A), mixed well.
Note: This solution is enough for two 96-well plates. The unused NAD/NADH mixture should be divided as single use aliquots and stored at -20 °C.

3. Prepare serial NADH (0 to 10 µM) solutions

3.1 Add 30 µL of 1 mM NADH standard stock solution (from step 1) to 970 µL PBS (pH 7.4) buffer to generate 30 µM (30 pmols/µL) standard.
Note: Diluted NADH standard solution is unstable, should be used within 4 hours.

3.2 Take 200 µL of 30 µM NADH standard solution (from step 3.1) to perform 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03 and 0 standard NADH solutions.

3.3 Add NADH standards and NAD/NADH containing test samples into a 96-well solid black microplate as described in Tables 1 and 2.
Note: Prepare your cell or tissue samples as desired. Component G (NAD/NADH Lysis Buffer) can be used for lysing the cells for convenience.

Table 1. *Layout of NADH standards and test samples in a solid black 96-well microplate:*

BL	BL	TS	TS	TS (NADH)	TS (NADH)	TS (NAD)	TS (NAD)				
NS1	NS1	….	….	….	….	….	….				
NS2	NS2										
NS3	NS3										
NS4	NS4										
NS5	NS5										
NS6	NS6										
NS7	NS7										

*Note: NS= NAD/NADH Standards; BL=Blank Control; TS=Test Samples; TS (NADH) = Test Samples treated with **NADH Extraction Solution** for 10 to 15 min, then neutralized by **NAD Extraction Solution**; TS (NAD) = Test Samples treated with NAD Extraction Solution for 10 to 15 min, then neutralized by **NADH Extraction Solution.***

Table 2. Reagent composition for each well:

NADH Standard	Blank Control	Test Sample (NAD/NADH)	Test Sample (NADH Extract)	Test Sample (NAD Extract)
Serial dilutions*: 25 µL	PBS: 25 µL	Test Sample: 25 µL	Test Sample: 25 µL	Test Sample: 25 µL
Component F: 25 µL	Component F: 25 µL	Component F: 25 µL	Component D: 25 µL	Component E: 25 µL
Incubate at room temperature for 10 to 15 min				
Component F: 25 µL	Component F: 25 µL	Component F: 25 µL	Component E: 25 µL	Component D: 25 µL
Total: 75 µL	Total: 75 µL	Total: 75 µL	Total: 75 µL	Total: 75 µL

**Note: Add the serially diluted NADH standards from 0.03 µM to30 µM into wells from NS1 to NS7 in duplicate. High concentration of NADH (e.g., >300 µM, final concentration) may cause reduced fluorescence signal due to the over oxidation of NADH sensor (to a non-fluorescent product).*

3.4 **For NADH Extraction (NADH),** add 25 µL NADH Extraction Solution (Component D) into the wells of NAD/NADH containing test samples. Incubate at room temperature for 10 to 15 minutes, then add 25 µL of NAD Extraction Solution (Component E) to neutralize the NADH extracts as described in Tables 1 & 2.

For NAD Extraction (NAD), add 25 µL NAD Extraction Solution (Component E) into the wells of NAD/NADH containing test samples. Incubate at room temperature for 10 to 15 minutes, then add 25 µL of NADH Extraction Solution (Component D) to neutralize the NAD extracts as described in Tables 1 & 2.

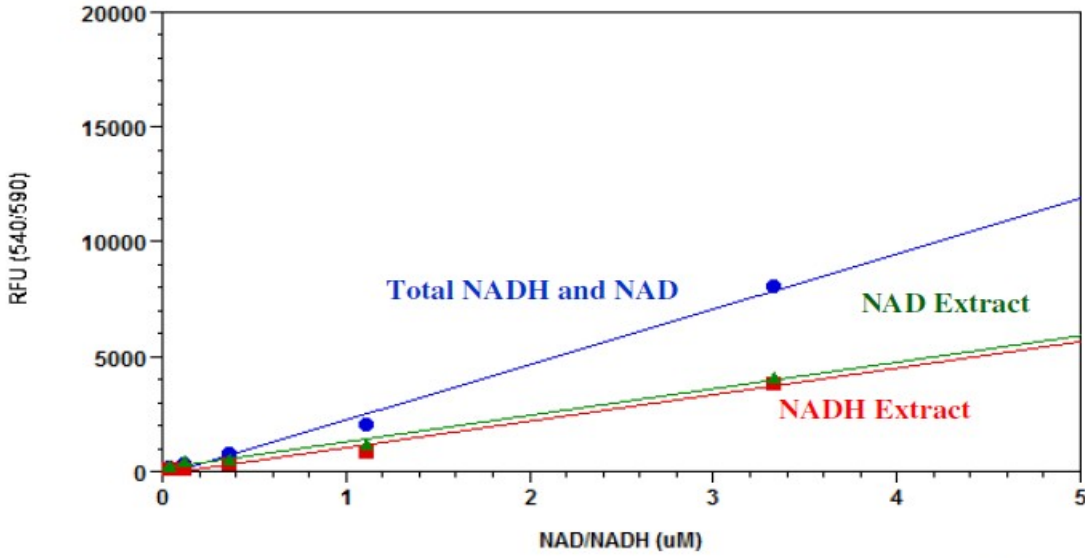
For Total NAD and NADH, add 25 µL NAD/NADH Control Solution (Component F) into the wells of NADH standards and NAD/NADH containing test samples. Incubate at room temperature for 10 to 15 minutes, and then add 25 µL of Control Solution (Component F) as described in Tables 1 and 2.
Note: Prepare your cell or tissue samples as desired. Component G (NAD/NADH Lysis Buffer) can be used for lysing the cells.

4. Run NAD/NADH assay in supernatants reaction

4.1 Add 75 µL of NADH reaction mixture (from step 2) to each well of the NADH standard, blank control, and test samples (from step 3.4) so that the total NADH assay volume is 150 µL/well.
4.2 Incubate the reaction for 15 minutes to 2 hours at room temperature, protected from light.
4.3 Monitor the fluorescence increase with Ex/Em = 540/590 nm using a fluorescence plate reader.
Note1: The contents of the plate can also be transferred to a white clear bottom plate and read by absorbance microplate reader at the wavelength of 576±5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

Data Analysis

The fluorescence in blank wells (with the PBS buffer only) is used as a control, and is subtracted from the values for those wells with the NADH reactions. The typical data are shown in Figure 1 (Total NAD and NADH vs. NAD or NADH Extract).



	y = A + Bx:	A	B	R^2
●	Plot#1 (Total NAD/NADH: Concentration vs Values)	-172.086	2411.885	0.993
■	Plot#2 (NADH Extract: Concentration vs Values)	-123.699	1154.729	0.987
▲	Plot#3 (NAD Extract: Concentration vs Values)	130.009	1155.929	0.992

Figure 1. Total NADH and NAD, and their extract dose response on 96-well black plate were measured with NAD/NADH Ratio Assay Kit using a Gemini microplate reader (Molecular Devices). 25 µL of equal amount of NAD and NADH was treated with or without NADH or NAD extraction solution for 15 minutes, and then neutralized with extraction solutions at room temperature. The signal was acquired at Ex/Em = 540/590 nm (cut off 570 nm) after 30 minutes of the addition of 75 µL of NADH reaction mixture. The blank signal was subtracted from the values for those wells with the NADH reactions *(Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point).*

References

1- Ziegenhorn J, *et al*. Molar absorptivities of beta-NADH and beta-NADPH. *Clin Chem*, 22, 151 (1976)

2- Ikegami T, *et al*. Structure and Properties of the Recombinant NADH-Cytochrome b(5) Reductase of Physarum polycephalum. *Biosci Biotechnol Biochem.* (2007)

3- Kimura N, *et al.* Comparison of metabolic fates of nicotinamide, NAD+ and NADH administered orally and intraperitoneally; characterization of oral NADH. *J Nutr Sci Vitaminol* (Tokyo), 52, 142 (2006)

4- O'Donnell JM, *et al.* Limited transfer of cytosolic NADH into mitochondria at high cardiac workload. Am J Physiol Heart Circ Physiol, 286, H2237 (2004)

Technical and scientific information

Related products

See BioSciences Innovations catalogue and e-search tool.

- Fluorimetric NADP/NADPH Assay Kit, JQ7300
- Fluorimetric NADH Assay Kit, JQ7320
- Fluorimetric NADPH Assay Kit, JQ7330
- Fluorimetric NADP/NADPH Ratio Assay Kit, ZE8130

Ordering information

Catalog size quantities and prices may be found at www.interchim.com/
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