Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

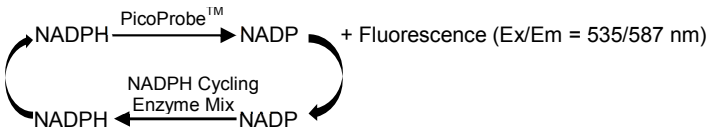
PicoProbe™ NADPH Quantitation Fluorometric Assay Kit

rev. 8/13

(Catalog # K349-100; 100 assays; Store at -20°C)

I. Introduction:

NADP (nicotinamide dinucleotide) is a major electron donor for anabolic reactions such as lipid and nucleic acid synthesis. The reduced form, NADPH is a cofactor for many redox enzymes. It is of increasing interest to be able to measure low level of intracellular NADPH. BioVision's PicoProbe™ NADPH Quantitation Assay kit provides a highly sensitive method to detect intracellular NADPH. NADH is not detected as the NADPH Recycling Enzyme Mix specifically recognizes NADPH in the enzyme recycling reaction. The assay is rapid, simple and does not require purifying NADPH from samples. The kit can measure less than 40 nM NADPH in a variety of samples.



II. Application:

- Measurement of NADPH in various tissues/cells
- Analysis of metabolism and cell signaling in various cells

III. Sample Type:

- Animal tissues: Liver, muscle, heart etc.
- Cell culture: Adherent or suspension cells
- Mitochondria

IV. Kit Contents:

Components	K349-100	Cap Code	Part Number
NADPH Extraction Buffer	50 ml	NM	K349-100-1
NADPH Cycling Buffer	15 ml	WM	K349-100-2
PicoProbe™	0.4 ml	Blue	K349-100-3
NADPH Cycling Enzyme Mix	0.2 ml	Green	K349-100-4
NADPH Standard (Lyophilized)	1 vial	Yellow	K349-100-5

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (Fluorescence reader)

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm NADPH Extraction Buffer & NADPH Cycling Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:

- **PicoProbe™:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **NADPH Cycling Enzyme Mix**: Ready to use as supplied. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months. Keep on ice while in use.
- **NADPH Standard:** Reconstitute with 200 µl DMSO to generate 1 mM (1 nmol/µl) NADPH Standard solution. Store at –20°C. Use within two months. Keep on ice while in use.

VIII. NADPH Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) on ice with 200 µl ice cold NADPH Extraction Buffer. Keep on ice for 10 min. Centrifuge at 10,000 X g for 5 min. Collect the supernatant. Add 1-50 µl sample into an eppendorf tube and bring the volume to 80 µl with NADPH Extraction Buffer.

   Note:

   a. For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

   b. For samples having high background, prepare parallel sample well(s) as sample background control.

2. **NADP Decomposition:** To detect NADPH, the NADP needs to be decomposed before the reaction. Keep samples at 60^{0}C for 30 min. to completely decompose the NADP. Cool samples on ice. Centrifuge briefly and transfer 50 µl of samples into a 96-well plate.

3. **Standard Curve Preparation:** Dilute NADPH Standard to 10 µM (10 pmol/µl) by adding 10 µl of 1 mM NADPH Standard to 990 µl NADPH Extraction Buffer, mix well. Dilute 10 µM NADPH Standard further to 1 µM (1 pmol/µl) by adding 50 µl of 10 µM NADPH to 450 µl NADPH Extraction Buffer, mix well. Add 0, 2, 4, 6, 8 & 10 µl of diluted 1 µM NADPH Standard into a series of wells in 96-well plate to generate 0, 2, 4, 6, 8, and 10 pmol/well NADPH Standards. Adjust the volume to 50 µl per well with NADPH Extraction Buffer.

   Note:

   Prepare working solution of NADPH Standard just before use (use within 4 hrs). Don't store the diluted Standard.

4. **Reaction Mix:** Prepare enough reaction mix for the number of assays (Standards, samples and background controls) to be performed. For each well, prepare 100 µl Reaction Mix containing:

	Reaction Mix	* Background Control Mix
NADPH Cycling Buffer	97 µl	99 µl
NADPH Cycling Enzyme Mix	2 µl	---
PicoProbe™	1 µl	1 µl

Add 100 µl of Reaction Mix to each well containing the Standards & samples, mix well.
* For samples having high background, add 100 µl of Background Control Mix to sample background control well(s). Mix well.

5. **Measurement:** Incubate the reaction for 60 min. at room temperature. Measure fluorescence (Ex/Em = 535/587 nm).
6. **Calculation:** Subtract 0 Standard reading from all readings. Plot the NADPH Standard Curve. If the sample background control reading is significant, subtract the background control reading from sample readings. Apply the corrected sample reading to the Standard Curve to get B pmol of NADPH in the sample wells.

Sample NADPH concentration (C) = B/V x Dilution Factor = pmol/µl = nmol/ml = µM

Where: **B** is the amount of NADPH from the Standard Curve (pmol)
V is the sample volume used in the reaction well (µl)

NADPH molecular weight: 744.413 g/mol

NADPH in Samples can also be expressed in pmol/mg.

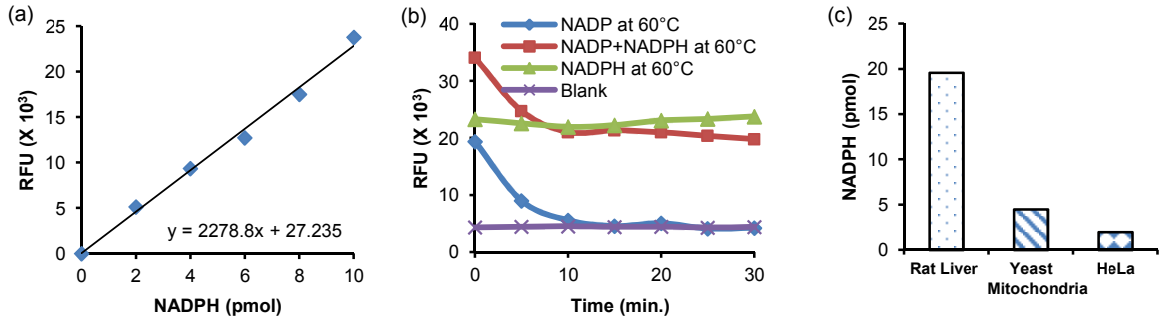


Figure: (a) NADPH Standard Curve, (b) Decomposition of NADP, but not NADPH at 60^{0}C & (c) Measurement of NADPH in rat liver lysate (150 µg), yeast mitochondrial lysate (150 µg) and HeLa lysate (150 µg). Assays were performed following kit protocol.

IX. RELATED PRODUCTS:

NAD/NADH Quantification Colorimetric Kit (K337)
PicoProbe™ NADH Fluorometric Kit (K338)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)
StayBrite™ Highly Stable ATP Bioluminescence Assay Kit (K791)
ApoSENSOR™ ADP/ATP Ratio Bioluminescence Assay Kit (K255)
NADP/NADPH Quantification Kit (K347)
ATP Colorimetric/Fluorometric Assay Kit (K354)
ADP Colorimetric/Fluorometric Assay Kit (K355)

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