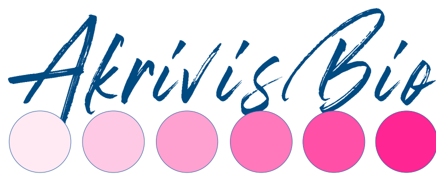


MA-0126

NADP/NADPH Ratio and Quantitation Assay

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Introduction:

NADP and NADPH play critical roles in metabolism and redox reactions. NADP acts as a coenzyme in various enzymatic reactions, particularly photosynthesis and oxidative stress defense. NADPH, the reduced form of NADP, is essential for biosynthetic reactions, such as fatty acid and nucleotide synthesis. Moreover, NADPH serves as a potent reducing agent, protecting cells from oxidative damage and maintaining redox homeostasis. The dynamic interplay between NADP and NADPH is indispensable for cellular function and overall metabolic balance. AkrivisBio's NADP/NADPH Assay is a simple, sensitive colorimetric method for measuring NADP and/or NADPH at the low to 100 picomole level in a wide range of biological samples.

Assay Principle:

1 – NADP present in a sample is used by an NADP specific alcohol dehydrogenase to form NADPH
2 – NADPH is used to reduce a nearly colorless tetrazolium salt to a highly colored formazan. The NADPH is converted back to NADP
3 – NADP in a sample can be selectively destroyed by incubation at an appropriate pH in a selected buffer without affecting NADPH

Kit Contents:

Extraction Buffer	50 ml	NM	MA-0126A
Cycling Buffer	15 ml	WM	MA-0126B
Alcohol Dehydrogenase	0.2 ml	Green	MA-0126C
WST Reagent	lyoph	Purple	MA-0126D
Stop Solution	1.2 ml	Red	MA-0126E
NADPH Standard	lyoph	Yellow	MA-0126F

User Supplied Materials:

- PBS
- DMSO
- 10 kDa MWCO centrifugal filters

Storage and Handling:

Store unopened kit at -20°C. Centrifuge all small vials for a few seconds before opening. Warm kit components to room temperature before use.
Extraction and Cycling Buffers: Ready to use as supplied. Store at 4°C
Alcohol Dehydrogenase: Keep on ice while in use. If the kit is to be used over a few weeks, aliquot into convenient portions. Store at -20°C.
WST Reagent: Reconstitute with 1.2 ml DI water. Let stand for 2 minutes then triturate to dissolve. Store at 4°C.
Stop Solution: Ready to use as supplied; Store at 4°C.
NADPH Standard: Dissolve in 200 µl DMSO. Store at 4°C.

Assay Protocol:

1. Standard Curve: Dilute the NADPH Standard to 0.4 µM (Transfer 10 µl to a tube containing 990 Extraction Buffer to give 4 pmol/µl NADPH. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the diluted NADPH Standard to a series of wells in a 96-well plate in duplicate to give 0, 20, 40, 60, 80 and 100 pmol/well. Adjust all well volumes to 50 µl with Extraction Buffer.

Notes: Diluted NADPH solution is unstable, must be used within a few hours. NADPH from disrupted cells or tissues is susceptible to degradation due to a variety of enzymes present. NADP/NADPH can be better preserved by filtering lysed samples as described below through a 10 kDa MWCO filter or by precipitating protein using one of our Sample Deproteinizing Kits. (make sure resulting solution is neutral to alkaline afterwards)

2. Sample Prep: Cells: Wash cells (4 x 10^6) or tissue (50 mg) with ice cold PBS. Pellet washed cells by spinning (2000 X g, 5 min). Remove supernatant and add 800 µl of Extraction Buffer, mix and place on ice for 10 minutes. Centrifuge at 16,000 X g and transfer supernatant to a fresh tube.
Tissue: Homogenize with 500 µl Extraction Buffer and place on ice for 10 min. Spin at 16,000 X g, 10 minutes and transfer supernatant to a fresh tube.
Either total (NADP + NADPH) or NADPH only can be determined using this assay.
Total: To detect total (NADP + NADPH) , transfer 50 µl of extracted samples to wells of a 96-well plate in duplicate.
NADPH only: To detect NADPH only, decompose NADP by transferring 200 µl of each sample to a microcentrifuge tube and heat at 60°C for 30 min. Under these conditions, NADP is selectively decomposed while. Cool on ice. Centrifuge samples briefly if precipitates are noted. Transfer 50 µl of each sample to wells in a 96-well plate in duplicate.

3. Initiate Reaction: Each well requires 100 µl of reaction mix. Prepare sufficient reaction mix for the number of wells to be run.

Cycling Buffer:	98 µl
Alcohol Dehydrogenase:	2 µl

Mix well and add 100 µl of the mix into each well. Let the plate sit undisturbed for 5 min to convert NADP to NADPH before initiating cycling reaction.

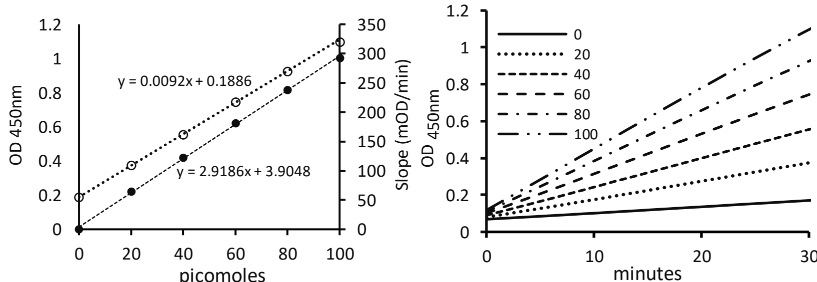
4. Add 10 µl WST Reagent to each well and mix.

5. Measurement: Monitor the reaction at 450 nm in kinetic mode for up to 4 hours at room temperature.

Note: This is a kinetic assay in which the rate of the reaction is proportional to the amount of NADPH present. The reaction can be stopped at any time, if desired, by addition of 10 µl Stop Solution to each well. The color is stable for 24-48 hours in a sealed plate, after the reactions are stopped.

6. Typical results:

picomole Standard	Standard Raw Values (OD)		Background Corrected Values (OD)	
0	0.1883	0.1880	0	0
20	0.3712	0.3777	0.1829	0.1897
40	0.5513	0.5588	0.3630	0.3708
60	0.7471	0.7407	0.5588	0.5527
80	0.9295	0.9212	0.7412	0.7332
100	1.1016	1.0912	0.9133	0.9032



7. Calculations: Subtract the 0 standard from all standards and samples using either the values at a fixed time point or by determining the slopes of the individual reactions. Examine the kinetic curves carefully and select a time range which only encompasses a linear increase in absorbance vs. time. As the absorbance increases, the rate of the reaction tends to decrease resulting in a downward curve of the reaction progress. Plot the standard curve as OD or slope vs. picomole of NADPH. Convexity at the upper end of the standard curve shows subtle nonlinearity and points not on a straight line should be excluded. Apply the slope from the standard curve to each sample by dividing the sample value by the slope of the standard curve.

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