NADP+/NADPH Quantification Kit

(Catalog #K347-100; 100 assays; Store kit at –70°C)

I. Introduction:

Assays of nicotinamide nucleotides are of continual interest in the studies of energy transforming and redox state of cells or tissue. The NADP/NADPH Quantification Kit provides a convenient tool for sensitive detection of the intracellular nucleotides: NADP, NADPH and their ratio. The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction (It does not recognize NAD+/NADH). There is no need to purify NADP/NADPH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity. Results can be quantified using plate reader at $OD_{450\ nm}$.

II. Kit Contents:

Components	K347-100	Cap Code	Part No.
NADP/NADPH Extraction Buffer	50 ml	NM	K347-100-1
NADP Cycling Buffer	15 ml	WM	K347-100-2
NADP Cycling Enzyme Mix	1 vial	Green	K347-100-3
NADPH Developer	1 vial	Purple	K347-100-4
Stop Solution	1.2 ml	Red	K347-100-5
NADPH Standard (MW:833.36)	166.7 µg	Yellow	K347-100-6

III. NADP/NADPH Assay Protocol:

A. Reagent Reconstitution and General Consideration:

- Reconstitute NADP Cycling Enzyme Mix with 220 µl NADP Cycling Buffer. Reconstitute NADPH developer with 1.2 ml of ddH2O. Pippet up and down several times to completely dissolve the pellet into solution. Aliquot enough NADP Cycling Enzyme mix (2 µl per assay) for the number of assays to be performed in each experiment and freeze the stock solution immediately at –70°C for future use. The reconstituted enzymes are stable for up to 2 months at –70°C.
- Reconstitute NADPH standard with 200 µl pure DMSO to generate 1 nmol/µl NADPH standard stock solution.
- Ensure that the NADP Cycling Buffer is at room temperature before use. The optimal temperature is 22°C. Keep other enzymes on ice during the assay and protect from light as much as possible.

B. Sample Preparation:

1. For cell samples*, wash cells with cold PBS. Pellet 10^5 cells for each assay in a microcentrifuge tube (2000 rpm for 5 min). Extract the cells with 200 µl of NADP/NADPH Extraction Buffer by freeze/thaw two cycles (20 min on dry-ice, then 10 min at room temperature), or homogenization. Vertex the extraction for 10 sec. Spin the sample at 14000 rpm for 5 min. Transfer the extracted NADP/NADPH solution into a new labeled tube.

2. For tissue samples*, weight ~20 mg tissue for each assay, wash with cold PBS, homogenize with 400 µl of NADP/NADPH Extraction Buffer in a microcentrifuge tube. Spin the sample at 14000 rpm for 5 min. Transfer the extracted NADP/NADPH solution into a new labeled tube.

   ***Note:** Cell or tissue lysates may contain enzymes that consume NADPH ralidly. We suggest to remove these enzymes from the sample by filtering the samples through 10 Kd molecular weight cut off filters (BioVision, Cat # 1997-25) before performing the assays.

C. NADP/NADPH Assay Protocol:

1. **Standard Curve:** Dilute 10 µl of the 1 nmol/µl NADPH standard with 990 µl NADP/NADPH Extraction Buffer to generate 10 pmol/µl standard NADPH (Note: diluted NADPH solution is unstable, must be used within 4 hours). Add 0, 2, 4, 6, 8, 10 µl of the diluted NADPH standard into labeled 96-well plate in duplicate to generate 0, 20, 40, 60, 80, 100 pmol/well standard. Make the final volume to 50 µl with NADP/NADPH extraction buffer.

   Samples: To detect total NADP/NADPH (NADPt), transfer 50 µl of extracted samples into labeled 96-well plate in duplicates. (Note: several sample dilutions should be performed to ensure the reading can be within the standard curve range.)

   Decompose of NADP from extraction: To detect NADPH only, aliquot 200 µl samples into eppendorf tubes. Heat samples to 60°C for 30 min in a water bath or a heating block. Under the conditions, all NADP will be decomposed while NADPH will still be intact. Cool samples on ice. Quick spin samples if precipitates occur.

   Transfer 50 µl of NADPH samples into labeled 96-well plate in duplicates (Note: several sample dilutions should be performed to ensure the reading can be within the standard curve range).

2. Prepare a NADP Cycling Mix for each reaction:

   NADP Cycling Buffer Mix: 98 µl
   NADP Cycling Enzyme Mix: 2 µl

   Mix well and add 100 µl of the mix into each well, mix well.

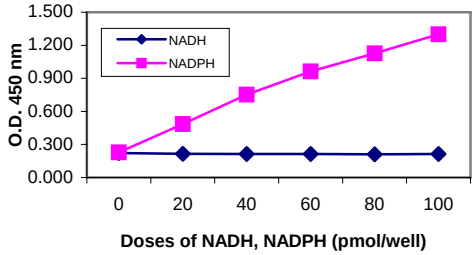
3. Incubate the plate at room temperature for 5 min to convert NADP to NADPH.

4. Add 10 µl NADPH developer into each well. Let the reaction develop for 1 to 4 hours. Read the plate at OD450 nm.

   Note: The signal increases as the reaction time. The plate can be read multiple times while the color is in developing. The reaction can be stopped by addition of 10 µl Stop Solution each well and mix well. The color should be stable within 48 hours in a sealed plate, after the reactions are stopped.

5. Calculation: Apply the sample OD450nm reading to standard curve. The background reading is very significant in the enzyme cycling reaction, therefore should be subtracted from standard and samples. The amount of NADPt or NADPH can be expressed in pmol/10^6 cells or ng/mg protein (NADPH molecular weight 833.36).

NADP/NADPH Ratio is calculated as: $\frac{\text{NADPt - NADPH}}{\text{NADPH}}$



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