NADP / NADPH Assay kit

ARG83367 NADP / NADPH Assay kit is an assay kit for NADP / NADPH in all biological

Catalog number: ARG83367

Package: 100 assay

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Nicotinamide adenine dinucleotide phosphate, abbreviated NADP+ is a cofactor used in anabolic reactions, such as the Calvin cycle and lipid and nucleic acid syntheses, which require NADPH as a reducing agent ('hydrogen source'). NADPH is the reduced form, whereas NADP+ is the oxidized form. NADP+ is used by all forms of cellular life.

NADP+:

In general, NADP+ is synthesized before NADPH is. Such a reaction usually starts with NAD+ from either the de-novo or the salvage pathway, with NAD+ kinase adding the extra phosphate group. ADP-ribosyl cyclase allows for synthesis from nicotinamide in the salvage pathway, and NADP+ phosphatase can convert NADPH back to NADH to maintain a balance.Some forms of the NAD+ kinase, notably the one in mitochondria, can also accept NADH to turn it directly into NADPH.The prokaryotic pathway is less well understood, but with all the similar proteins the process should work in a similar way.

NADPH:

NADPH is produced from NADP+. The major source of NADPH in animals and other non-photosynthetic organisms is the pentose phosphate pathway, by glucose-6-phosphate dehydrogenase (G6PDH) in the first step. The pentose phosphate pathway also produces pentose, another important part of NADPH, from glucose. Some bacteria also use G6PDH for the Entner–Doudoroff pathway, but NADPH production remains the same.

PRINCIPLE OF THE ASSAY

ARG83367 NADP / NADPH Assay Kit measures NADP and NADPH content in biological samples. This assay kit reduced NADP+ to NADPH, add probe, measured at 450 nm. Compared the samples to NADP+ standard within the 96-well microtiter plate format.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard and NADP Cycling Enzyme at -80°C, store Probe at -20°C. Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
NADP+ Standard (20 mM)	50 µL	-80°C
10X Extraction Buffer	10 ml	4°C
Probe	1 ml	-20°C
NADP Cycling Enzyme	15 µL	-80°C
NADP Cycling Substrate	50 µL	4°C
Assay Buffer	25 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Flat bottomed 96-well black microplate
- 10 kDa molecular weight centrifuge spin filter
- Pipettes and pipette tips
- PBS, 0.5 N H2SO4 and 0.1 N NaOH / HCl

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Store Standard and NADP Cycling Enzyme at -80°C, store Probe at -20°C. Store other component at 2-8°C at all times. Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Saliva- Collect saliva using a collection device (e.g. Salivette), centrifuge 10,000 x g for 2 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles. The collection device should not have protein binding or filtering features.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at-80°C. Perform dilutions in PBS.

REAGENT PREPARATION

- **1X Extraction Buffer:** Dilute the 10X Extraction Buffer with deionized water to yield 1X Extraction Buffer. Store at 4°C.
- **NADP Cycling Reagent:** Dilute the NADP Cycling Substrate at 1:100, NADP Cycling Enzyme at 1:400, and Colorimetric Probe at 1:5 in 1X Assay Buffer. For 100 assays, mix e 50 µL NADP Cycling Substrate, 12.5 µL NADP Cycling Enzyme, 1000 µL Probe to 3.938 mL of 1X Assay Buffer for total volume of 5 mL.
- **NADPH Extraction Procedure:** To measure NADPH and destroy NADP+, add 25 µL of sample to a microcentrifuge tube. Add 5 µL of 0.1 N NaOH and mix thoroughly. Incubate the tube at 80°C for 60 minutes and protected from light. Centrifuge the tube to pool all sample solution. Add 20 µL of 1X Assay Buffer to shift the pH of the sample back to neutral. Vortex to mix and centrifuge to pool sample. Sample pH should be between 6.0 and 8.0; if not, neutralize accordingly with acid or base. Keep sample on ice until assaying.
- **NADP+ Extraction Procedure:** To measure NADP+ and destroy NADPH, add 25 µL of sample to a microcentrifuge tube. Add 5 µL of 0.1 N HCl and mix thoroughly. Incubate the tube at 80°C for 60 minutes and protected from light. Centrifuge the tube to pool all sample solution. Add 20 µL of 1X Assay Buffer to shift the pH of the sample back to neutral. Vortex to mix and centrifuge to pool sample. Sample pH should be between 6.0 and 8.0; if not, neutralize accordingly with acid or base. Keep sample on ice until assaying.

- **Standards**: Add 5 µl of 20 mM stock standard into 995 µl 1X Extraction to yield **100 µM stock 1** Buffer. Add 5µl of **100 µM stock 1** into 495 µl 1X Extraction to generate a standard with 1 µM of NADP+. Dilute the standards with Extraction Buffer serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard	NADP+ (µM)	Volume of 1X Extraction Buffer (µL)	Volume of NADP + (µL)
stock 1	100	995	5 (20 mM stock)
S1	1	495	5 (100 µM stock 1)
S2	0.5	250	250(S1)
S3	0.25	250	250(S2)
S4	0.125	250	250(S3)
S5	0.063	250	250(S4)
S6	0.031	250	250(S5)
S7	0.015	250	250(S6)
S8	0.008	250	250(S7)
S9	0.004	250	250(S8)
S10	0	250	0

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **50 µl** of **standard** and **sample** to each wells.
2. Add **50 µL** of **NADP Cycling Reagent** each wells.
3. Mix well and Incubate for 1-4 hour at RT, protected from light.

   Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics. The assay may be stopped at a desired time point by adding 50 µL 0.5 N H_2SO_4.
4. Read O.D. with a microplate reader at 450 nm

CALCULATION OF RESULTS

1. Calculate the average fluorescence values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected background fluorescence. If sample background control value is high, subtract the sample background control value from the sample reading.
2. Plot the corrected fluorescence for the NADP+ standards against the final concentration of the standards.
3. Since all NADP+ is converted to NADPH by the Cycling Reagent, use the standard curve to determine the total NADP+ / NADPH concentration in pmoles within the sample. Determine the total concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected fluorescence values for each sample. Remember to account for dilution factors.

   Total NADP+ /NADPH =

   (Corrected sample fluorescence / Slope) X N

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of NADP / NADPH ranged from 0.78-100 nM. The mean MDD was 0.4 nM