NADPase Assay Kit

NADPase Assay Kit is a detection kit for the quantification of NADPase Activity in tissue extracts and cell lysate.

Catalog number: ARG82018

Package: 96 wells

TABLE OF CONTENTS

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INTRODUCTION

Nicotinamide adenine dinucleotide phosphate, abbreviated $NADP^+$ or, in older notation, TPN (triphosphopyridine nucleotide), 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. It is used by all forms of cellular life.

NADPH is the reduced form of $NADP^+$. $NADP^+$ differs from NAD^+ by the presence of an additional phosphate group on the 2' position of the ribose ring that carries the adenine moiety. This extra phosphate is added by NAD^+ kinase and removed by $NADP^+$ phosphatase. [Provide by Wikipedia: Nicotinamide adenine dinucleotide phosphate]

PRINCIPLE OF THE ASSAY

This NADPase Assay Kit is a simple colorimetric assay that measures the amount of NADPase present in tissue extracts and cell lysate. The assay is based on the enzyme driven reaction. NADPase can catalyze the NAD^+ hydrolysis to $NADP^+$ and reactions of inorganic phosphorus, NADPase activity was measured by measuring the amount of inorganic phosphorus. The concentration of NADPase in the samples is then determined by comparing the O.D. 660 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well microplate	1 plate	RT
Assay Buffer	4 x 30 mL (ready to use)	4°C
Reaction Buffer	20 mL (ready to use)	4°C
Substrate (lyophilized)	2 vial	-20°C
Dye Reagent I (lyophilized)	1 vial	4°C
Dye Reagent II (lyophilized)	1 vial	4°C
Dye Reagent III	20 mL	4°C
Standards (5 µmol/mL)	1 mL	4°C
Plate sealer	3 ea	RT
Technical Manual	1 ea	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 660 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice, centrifuged at 8,000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

REAGENT PREPARATION

- **Substrate:** Add 1 mL of Reaction Buffer before use.
- **Dye Reagent:** Add 1 mL of Dye Reagent III into Dye Reagent I and Dye Reagent II respectively for dissolve, then mix 3 Dye Reagent together.

Note: Final Dye reagent should be yellow. If colorless, the solution is failure. If blue, the solution is polluted. This solution should be prepared before use. It is best to use disposable plastic containers to prepare the solution in order to prevent phosphorus pollution.

- **Standards:** Use the 5 µmol/mL Standards to prepare a series of diluted standards according to the Table below.

Standard tube	Final Standard conc. (µmol/mL)	distilled water (µL)	Volume of 5 µmol/mL Standards (µL)
S1	5	0	500
S2	2.5	250	250 of S1
S3	1.25	250	250 of S2
S4	0.625	250	250 of S3
S5	0.313	250	250 of S4
S6	0.156	250	250 of S5
S7	0.078	250	250 of S6
S0	0	250	0

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Add following reagents into the microcentrifuge tubes:				
Reagent	**Sample**	**Control**	**Standards**	**Blank**
Reaction Buffer	140 µL	140 µL		
Substrate	20 µL	20 µL		
Mix well, and incubate for **5 minutes** at **37°C**.				
Sample	40 µL			
Distilled water		40 µL		
Mix well, and incubate for **30 minutes** at **37°C**. Then put in **boiling water** for **5 minutes**. When cold, centrifuged at **10,000 x g** for **5 minutes** at **room temperature**.				
Add following reagent into 96 Well microplate:				
Standards			20 µL	
Distilled water				20 µL
Supernatant	20 µL	20 µL		
Dye reagent	180 µL	180 µL	180 µL	180 µL
Mix well, and incubate for **30 minutes** at **room temperature**. Read the absorbance at **O.D. 660 nm**.				

Note: It is best to use disposable plastic tube to avoid phosphorus pollution.

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Unit Definition: One unit of NADPase activity is defined as the enzyme generates 1 µmol of PO_4^{3-} per hour.

5. According to the protein concentration of sample:

NADPase (U/mg)

$= \{[(C_{Standaard} \times V_{Total}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / [(V_{Sample} \times C_{Protein})\} / T$

$= [50 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / C_{Protein}$

6. According to the weight of sample:

NADPase (U/g)

$= \{[(C_{Standaard} \times V_{Total}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / [(V_{Sample} \times W / V_{Assay})\} / T$

$= [50 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})] / W$

Note:

$C_{Protein}$: the protein concentration of sample, mg/mL;

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 5 μmol/mL;

V_{Total}: the total volume of the enzymatic reaction, 0.2 mL;

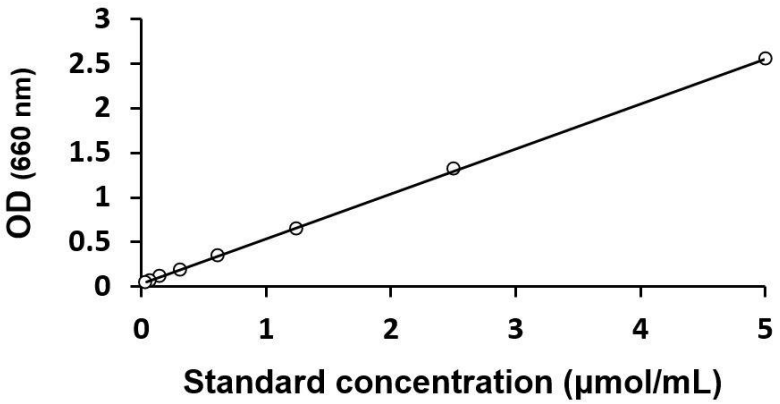
V_{Sample}: the volume of sample, 0.04 mL;

V_{Assay}: the volume of Assay buffer, 1 mL;

T: the reaction time, 0.5 hour.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the NADPase Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.01 µmol/mL