CheKine™ Micro Coenzyme II NADP(H) Assay Kit

Cat #: KTB1010 Size: 48 T/96 T

	Micro Coenzyme II NADP(H) Assay Kit		
REF	**Cat #:** KTB1010	LOT	**Lot #:** Refer to product label
	Detection range: 0.5 μM-10 μM		**Sensitivity:** 0.5 μM
	Applicable samples: Animal Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Nicotinamide adenine dinucleotide phosphate (NADP) is an enzymatic cofactor involved in many redox reactions where it cycles between the reduced (NADPH) and oxidized (NADP) forms. $NADP^+$ is also involved in biosynthetic reactions such as lipid and nucleic acid synthesis where it functions as a reducing agent. The oxidative branch of the pentose phosphate pathway (PPP) is the major source of NADPH produced in animal cells. There is continual interest in monitoring their concentration levels. Quantitative determination of $NADP^+$/NADPH has applications in research pertaining to energy transformation and redox state of cells or tissues. CheKine™ Micro Coenzyme II NADP(H) Assay Kit provides a convenient tool for sensitive detection of the NADP and NADPH, and their ratio in various tissues and subcellular organelles. This Kit is based on enzyme cycling reaction (It does not recognize $NADP^+$/NADPH), in which the formed NADPH reduces a formazan (WST-8) reagent. The intensity of the reduced product color, measured at OD450 nm, is proportionate to the $NADP^+$/NADPH concentration in the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Assay Buffer	7.5 mL	15 mL	4℃
Glucose (1 M)	1 mL	2 mL	4℃
WST-8	300 μL	600 μL	-20℃, protected from light
Enhancer	60 μL	120 μL	-20℃, protected from light
NADP Cycling Enzyme Mix	60 μL	120 μL	-20℃, protected from light
NADPH Standard (10 mM)	100 μL	200 μL	-20℃, protected from light
NADP Extraction Buffer	6 mL	12 mL	4℃

NADPH Extraction Buffer	6 mL	12 mL	4℃

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at OD450 nm
- Incubator
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Note: Briefly centrifuge small vials at low speed prior to opening.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Glucose (1 M): Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

WST-8: Ready to use as supplied. Keep on ice protected from light during the assay. Store and aliquot the surplus reagent at -20℃, protected from light.

Enhancer: Ready to use as supplied. Keep on ice protected from light during the assay. Store aliquots at -20℃, protected from light.

NADP Cycling Enzyme Mix: Ready to use as supplied. Keep on ice protected from light during the assay. Store aliquots at -20℃, protected from light.

NADPH Standard: Ready to use as supplied. Keep on ice protected from light during the assay. Store aliquots at -20℃, protected from light.

NADP Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

NADPH Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent: Ready to use for each well of reaction to prepare 85 μL Working Reagent by mixing 68 μL Assay Buffer, 1 μL NADP Cycling Enzyme Mix, 5 μL WST-8, 1 μL Enhancer. The mixtures were preincubated 5 min at room temperature then add 10 μL Glucose. Working Reagent is freshly prepared.

Calibration NADPH Curve: Prepare 1 mL 10 μM NADPH Premix by mixing 1 μL 10 mM Standard and 999 μL Assay Buffer. Dilute standard as follows:

Num.	10 μM NADPH Premix (μL)	Assay Buffer (μL)	Volume (μL)	NADPH (μM)
Std1.	100	0	100	10
Std2.	80	20	100	8
Std3.	60	40	100	6
Std4.	40	60	100	4
Std5.	20	80	100	2
Std6.	10	90	100	1
Std7.	5	95	100	0.5

Sample Preparation

1. Conventional sample preparation

For Cell (adherent or suspension) samples: Harvest the number of cells necessary for each assay (initial recommendation=1×10^6 cells/assay). Wash cells with cold PBS. Pellet cells in a tube by spinning at low speed for 5 min, and discard supernatant.

For Tissues: Weigh the amount of tissue necessary for each assay (initial recommendation=20 mg). Wash with cold PBS. Cut

into pieces with scissors and place in a homogenizer.

Extract the NADP/NADPH: Homogenize samples (either tissue or cells) with either 100 µL NADP Extraction Buffer for NADP determination or 100 µL NADPH Extraction Buffer for NADPH determination. Heat extracts at 60℃ for 5 min and then add 20 µL Assay Buffer and 100 µL of the opposite Extraction Buffer (another Extraction Buffer) to neutralize the extracts. Briefly vortex and spin the samples at 14,000 rpm for 5 min. Transfer the extracted NADP/NADPH supernatant into a new labeled tube and keep on ice for NADP/NADPH assays. Determination of both NADP and NADPH concentrations requires extractions from two separate samples.

2. Bacteria sample preparation

For Bacteria samples: Harvest the amount of bacteria necessary for each assay (initial recommendation=1×10^6 bacteria/assay). Wash bacteria with cold PBS, pellet cells in a tube by spinning at low speed for 5 minutes, and discard supernatant. Add 100 µL NADP extraction buffer or NADPH Extraction Buffer, ultrasonic crushing was performed (20% strength or 200 W, ultrasonic for 3 s, stopping for 10 s, repeating 30 times), and Heat extracts at 60℃ for 5 min (cover tightly to prevent water loss), and then add 20 µL Assay Buffer and 100 µL the opposite Extraction Buffer (another Extraction Buffer) to neutralize the extracts and mix well. After cooling in the ice bath, the samples were centrifuged at 14,000 rpm at 4℃ for 5 min, take the supernatant and place it on ice for measurement.

Note: We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze Cells or Tissues in liquid nitrogen upon extraction and store the samples immediately at -80℃. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected. For additional measurement, it is recommended to use Abbkine Protein Quantification Kit (BCA Assay) (Cat #: KTD3001).

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 450 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (µL)	Standard Well (µL)	Test Well (µL)
Assay Buffer	40	0	0
Stds.	0	40	0
Sample	0	0	40
Working Reagent	80	80	80

3. Mix well. The absorbance values at 450 nm were measured as A_1; Incubate at room temperature for 30 min, and the record the absorbance values at 30 min as A_2. Calculate $\Delta A=A_2-A_1$, $\Delta\Delta A_{Standard}=\Delta A_{Standard}-\Delta A_{Blank}$, $\Delta\Delta A_{Test}=\Delta A_{Test}-\Delta A_{Blank}$.

Note: This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended; The following substances interfere and should be avoided in sample preparation. EDTA (>0.5 mM), ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta\Delta A_{Standard}$ as the x-axis, draw the standard curve. Use the $\Delta\Delta A_{Test}$ values to determine sample NADP/NADPH concentration y from the standard curve.

Note: If the $\Delta\Delta A_{Test}$ value of the sample is higher than the $\Delta\Delta A_{standard}$ value of the 10 µM standard, the sample should be

diluted with deionized water before the experiment.

2. Calculation of NADP/NADPH content

(1) By sample fresh weight

NADP/NADPH (nmol/g) =(y×V_{Sample})÷(W×V_{Sample}÷$V_{Sample\ Total}$)×n**=0.22×y÷W×n**

(2) By protein concentration

NADP/NADPH (nmol/mg prot)=(y×V_{Sample})÷(V_{Sample}×Cpr)×n**=y÷Cpr×n**

(3) By number of cells or bacteria

NADP/NADPH (nmol/10^4)=(y×V_{Sample})÷(number×V_{Sample}÷$V_{Sample\ Total}$)×n**=0.22×y÷number×n**

Where: nmol ,1μM=1 nmol/mL; V_{Sample}: add sample volume, 0.04mL; $V_{Sample\ Total}$: Total volume of sample, 0.22mL; Cpr: The protein concentration of the sample, mg/mL; W: Weight of sample, g; Number: the number of cells or bacteria, 10^4 as the unit; n: The sample dilution factor.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

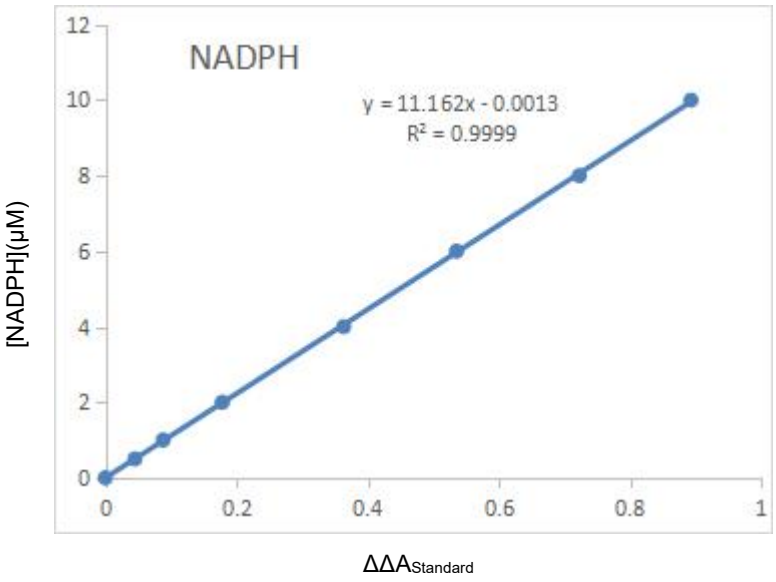

Figure 1. Standard curve of NADPH

Note: At these concentrations, the standard curves for NADP and NADPH are identical. We provide only NADPH as the standard.

Recommended Products

Catalog No.	Product Name
KTB1020	CheKine™ Micro Coenzyme Ⅰ NAD(H) Assay Kit
KTB1021	CheKine™ Micro NADH Oxidase (NOX) Assay Kit
KTB1022	CheKine™ Micro NAD^+ Kinase (NADK) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.