BQC

REDOX TECHNOLOGIES

KB03034

NADP+/NADPH Assay Kit

96 well plate
100/200/400 tests

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1. General information

PRECAUTIONS

Please read this manual carefully before beginning the assay.

This product is designed for **research use only**. It is not approved for human or animal use or clinical diagnosis. All chemicals should be handled with care and in accordance with laboratory safety practices. It is recommended to use basic Personal Protective Equipment.

Do not use after the expiration date stated on the packaging.

Do not mix or substitute reagents or materials from other kit batches or vendors.

For **the material safety data sheet (MSDS)** please contact as at info@bioquochem.com

TECHNICAL RECOMMENDATIONS

Store reagents as indicated in **Materials and storage** section.

Be sure to keep the bottle capped when not in use.

Let the components reach room temperature (RT) before use.

Immediately before use, gently invert and rotate reagent bottles several times to mix the contents thoroughly.

Avoid foaming or bubbles when mixing or reconstituting components.

Avoid cross contamination of samples or reagents by changing pipette tips between sample, standard and reagent additions.

Be sure to use the optimal microplate for the assay. Transparent microplates for UV/VIS applications, and black microplates for fluorescence measurements.

2. Technical specifications

Available sizes

100/200/400 tests

Required sample volume

100 µL/test

Compatible samples

Tissue homogenates and cell lysates

Type of detection

Colorimetric (570 nm)

3. Materials and storage

MATERIALS SUPPLIED

Item	No. Tests	Units	Storage
Solvent	100	1	4 °C
	200	2	
	400	4	
Extraction Buffer	100	1	4 °C
	200	2	
	400	4	
Reagent A	100	1	4 °C
	200	2	
	400	4	
Reagent B	100	1	4 °C
	200	2	
	400	4	
Reagent C	100	1	4 °C
	200	2	
	400	4	
Reagent D	100	1	-20 °C
	200	2	
	400	4	
Standard	100	1	-20 °C
	200	2	
	400	4	
Transparent 96-Well Microplate	100	1	RT
	200	2	
	400	4	

MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH_2O) as Milli-Q Ultrapure Water.
- Labware materials (micropipettes, tubes, stirring/mixing equipment).
- Colorimetric microplate reader – equipped with filter for OD 570 nm.

STORAGE CONDITIONS

On receipt, store kit components as indicated above. Under these conditions, the reagents are stable in the original packaging until the expiration date indicated on the outside of the box. After reconstitution, standard solutions are unstable in the presence of oxygen. Prepare a fresh set of standards for every use.

4. Introduction

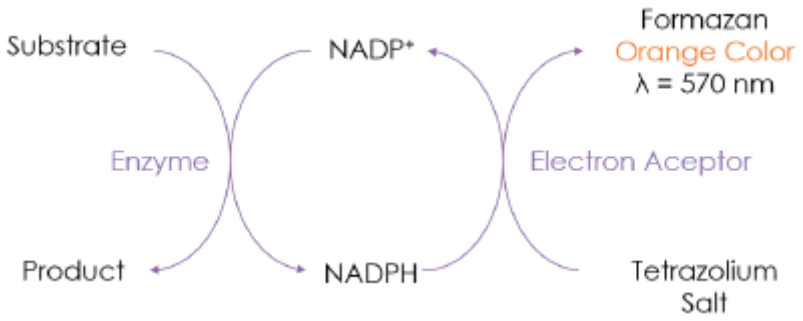
Nicotinamide adenine dinucleotide phosphate NADP(H) is a well-known coenzyme found in all living cells that plays an important role in energy metabolism, biosynthesis and detoxification. NADP(H) exists in cells in oxidized ($NADP^+$) and reduced (NADPH) forms reflecting the redox state of the cell. Prolonged disequilibrium of $NADP^+$/NADPH balance disturbs the physiological functions, resulting in several diseases like diabetes, cardiovascular diseases, neurodegeneration disorders or cancer.

BQC $NADP^+$/NADPH Assay Kit is a quick, easy, and reproducible assay for determining $NADP^+$/NADPH concentration in cells and tissues.

5. Assay principle

The BQC $NADP^+$/NADPH Assay Kit is based on an enzymatic cycling reaction in which $NADP^+$ is reduced to NADPH. The NADPH present in the sample and the newly formed NADPH are then oxidized by an electron intermediate acceptor resulting in the reduction of a tetrazolium salt to formazan. Formazan is a highly colored compound that shows a maximum absorption at 570 nm. The amount of formazan generated is proportional to the total amount of NADP(H) ($NADP^+$ + NADPH) in the sample.

The native NADPH can be determined alone by heating the sample (thermal degradation of $NADP^+$) before the assay.



Principle of $NADP^+$/NADPH Assay Kit

6. Assay preparation

REAGENT PREPARATION

All assay reagents not listed below are ready to use as supplied. Allow the reagents to reach room temperature before use.

Reaction Buffer: Add 30 mL of Solvent to the Reagent A bottle and mix thoroughly.

R.B. Working Solution: Add 100 µL of Solvent in each Reagent B vial and mix thoroughly. Dilute this solution 1:100 with Solvent (e.g. 10 µL of the previous solution with 990 µL of Solvent).

Enzyme Solution: Add exactly 1080 µL of Solvent to the Reagent C vial. Keep Enzyme Solution on ice until use.

R.D. Working Solution: Add 5 mL of Solvent to the Reagent D bottle and mix thoroughly.

Reagent Mix: Add 1.2 mL of Enzyme Solution and 800 µL of R.B. Working Solution to 20 mL of Reaction Buffer.

> **CAUTION:** R.B. Working Solution, Enzyme Solution, R.D. Working Solution and Reagent Mix must be prepared immediately before use.

Standard Solution (NADP+): Add 1 mL of Extraction Buffer to the Standard vial and mix well. Dilute this standard solution 1:100 with Extraction Buffer (e.g. 10 µL of standard solution with 990 µL of Extraction Buffer). Dilute this solution again 1:100 (e.g. 10 µL of standard solution with 990 µL Extraction Buffer). Use this 1:10000 diluted solution to prepare the standard curve.

STANDARD CALIBRATION

Prepare NADP+ standards for the calibration curve from the 1:10000 diluted Standard solution according to the following Table. Prepare the standards immediately prior to each assay. Vortex tubes thoroughly. Discard standard solutions after use.

Standard	Standard solution 1:10000 diluted (µL)	Extraction Buffer (µL)	NADP+ (nM)
Std 1 (Reagent Blank)	0	400	0
Std 2	20	380	50
Std 3	40	360	100
Std 4	80	320	200
Std 5	160	240	400
Std 6	200	200	500

PLATE SET UP

BQC recommends running the standards and samples at least in duplicate (triplicate recommended). There is no specific pattern for using the wells on the plate. A proposed layout of standards (Std) and samples (S) to be measured in duplicate is shown below.

NOTE: If sample blanks are included in the assay, it is necessary to reserve some wells of the plate for these blanks.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
B	Std 2	Std 2	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
C	Std 3	Std 3	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
D	Std 4	Std 4	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
E	Std 5	Std 5	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
F	Std 6	Std 6	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
G	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
H	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42

Example of plate layout for the NADP+/NADPH Assay Kit

7. Sample preparation

The following sample preparation protocols are intended as a guide only. The optimal conditions for sample preparation must be determined by the end user. It is recommended to use fresh samples. If it is not possible, aliquot and store samples properly with minimal freeze/thawing cycles.

NADP+/NADPH Assay Kit can be used to determine NADP(H) in tissue homogenates or cell lysates.

It is recommended to perform NADP+/NADPH Assay on deproteinized samples. Enzymes present in the samples may consume NADPH rapidly. Samples can be deproteinized by filtering through a 10 kDa cut-off spin filter.

Tissue Homogenates. Dissect the tissue of interest and place it on a homogenizer tube with an appropriate amount of ice-cold extraction buffer (i.e. 50 mg tissue per 0.5 mL extraction buffer). Homogenize the tissue and then centrifuge the homogenate at 10000 x g for 15 minutes at 4 °C and collect the supernatant.

Cell culture. Wash cells with ice-cold buffer before lysis. Pellet the cells by centrifugation ($4 \cdot 10^6$ cells recommended). Extract cells with 800 µL of Extraction Buffer. Place on ice for 10 minutes. Centrifuge the samples at 10000 x g for 10 minutes at 4 °C to remove insoluble material. Collect the supernatant.

Reagents and materials required for sample preparation are not supplied with the kit. Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

Make sure that interfering substances present in the sample do not give a significant background. Run proper blanks as necessary (e.g. sample blank should be always evaluated when working with highly colored samples). It is recommended to assay different sample dilutions to ensure the values fall within the standard curve.

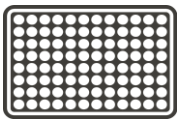
8. Assay protocol

Prepare and mix all reagents thoroughly before use. Each standard, sample or blank should be assayed at least in duplicate.

It is possible to determine both **total NADP(H)** (NADP+ + NADPH) and **NADPH** in each sample.

A. Total NADP(H) (NADP+ + NADPH). Leave the sample as it is and follow the protocol described above. If necessary, dilute the sample with the extraction buffer.

B. NADPH. Decompose the NADP of the extracted sample before the analysis. Aliquot 400 µL of the sample and heat to 60 °C for 30 minutes in a water bath or heating block. Cool samples on ice. Spin samples and remove any precipitates. Then, follow the protocol described above. If necessary, dilute the sample with the extraction buffer.

1  Set up the plate design

2 Add **100 µL** of **standard** or **sample** in each well

3 Add **50 µL** of **R.D. Working Solution** in each well

4 Add **170 µL** of **Reagent Mix** in each well

5 Incubate the plate during **15 minutes** at **RT**

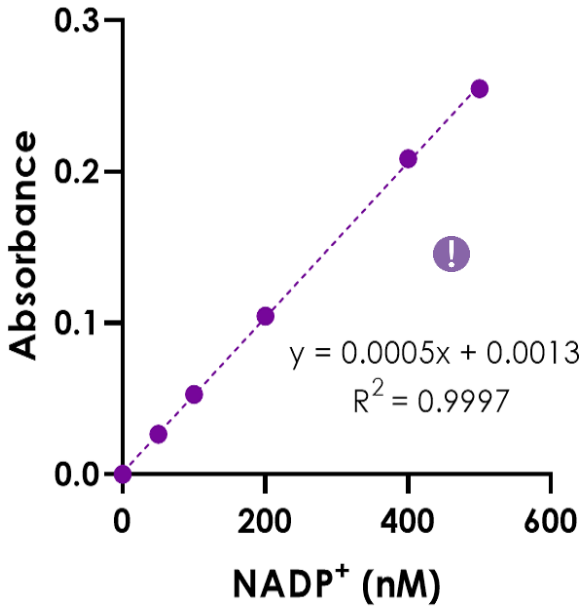
6 Read the **absorbance** of all wells at **570 nm** in end point mode at **RT**

If you need to **adapt this kit** for another form of the assay (for example cuvette), **contact us at** info@bioquochem.com

9. Data analysis

ANALYSIS OF THE STANDARDS

- Calculate the average absorbance of all standards.
- Subtract the average absorbance of the reagent blank (Std 1) from the average absorbance of all the standards to obtain the blank-corrected absorbance of the standards.
- Create a standard curve by plotting the blank-corrected absorbance of the standards as a function of the standard concentration (see **STANDARD CALIBRATION** section). A typical standard curve (**y**=slope·**x** ± intercept) for this assay is shown below.



NADP(H) standard curve with NADP+/NADPH Assay Kit

This standard curve is an example of the data typically obtained with this kit. **DO NOT USE** this standard curve to calculate the NADP(H) of your samples. A new standard curve must be performed by the end user.

ANALYSIS OF THE SAMPLES

- Calculate the average absorbance of the samples.
- Subtract the average absorbance of the reagent blank (Std 1) from the average absorbance of each sample to obtain the blank-corrected absorbance of the samples (A_S).
- Calculate the NADP(H) value of the samples using the following equation. Slope and intercept values are obtained from the standard curve.

$$\text{NADP(H) (nM)} = \left(\frac{A_S - \text{intercept}}{\text{slope}}\right)$$

When working with diluted samples the NADP(H) values obtained must be multiplied by the dilution factor to obtain the NADP(H) value of the undiluted sample.

The results obtained depend on the assay format:

Total NADP(H): concentration value obtained for the **total NADP(H) assay**.

NADPH: concentration value obtained for the **NADPH assay**.

NADP+: concentration obtained by **subtracting** the concentration calculated for **NADPH from the total NADP(H)** concentration.

10. Troubleshooting

This troubleshooting table provides potential sources and solutions for common problems observed with BQC Assay Kits. **The problems listed below could occur when using any BQC Assay Kit**. They are not specific for this assay Kit.

Problem	Possible Cause	Recommended Solution
Wells have color but there is no reading	Plate read at incorrect wavelength	Check the wavelength used in the assay
	Incorrect microplate	Use the correct microplate for your application UV/Vis: transparent Fluorescence: black wells/transparent bottom
Standard readings do not follow a linear pattern	Pipetting errors in preparation of standards	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique
	Standard stock is at incorrect concentration	Always refer to dilutions described in **Assay preparation**
	Improperly thawed reagents	Thaw all components completely and mix well before use
	Use of improperly stored reagents	Store the components appropriately Use fresh components from the standard curve
	Incorrect incubation times or temperatures	Refer to **Assay protocol**
Dispersion of standard and sample readings	Pipetting errors	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique

Problem	Possible Cause	Recommended Solution
Sample erratic values	Samples contain interfering substances	Dilute sample further (if possible)
	Inappropriately stored samples or samples used after multiple freeze-thaw cycles	Use fresh samples or store appropriately until use
	Samples not deproteinized	Use an appropriate deproteinization protocol
	Cells/Tissue samples not homogenized completely	Repeat the sample homogenization
	Inappropriate sample dilution buffer	Refer to **Assay preparation**
Sample reading fall outside the detection range	Samples are too diluted/concentrated No analyte/activity is observed in the sample	Re-assay using different sample dilutions

STILL HAVING PROBLEMS?

Contact BQC if you have any further questions, our team will be pleased to help you:

	Phone	+ 34 985 26 92 92
	E-mail	info@bioquochem.com
	Business hours	Monday-Thursday: 8.30 to 17.00 (CEST) Friday: 8.00 to 15.00 (CEST)

11. Additional information

NADP/NADPH determination Assay Kit is a quick (< 30 minutes) and highly reproducible sensitive assay for determining NADP(H) in a wide variety of samples.

Detergents (>5%) and other substances with a maximum absorbance at 570 nm have been reported to interfere with this assay.

If unexpected results are obtained running your samples, please contact us at info@bioquochem.com

12. Related products

More products available on bioquochem.com

Reference	Product
KB03033	NAD/NADH Quantification Assay Kit
KB03007	Thiol Quantification Assay Kit
KB03032	Xanthine Oxidase Activity Assay Kit

13. Warranties and limitation of liability

BQC shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if BQC has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, downtime, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by BQC´s gross negligence. Any and all liability of BQC hereunder shall be limited to the amounts paid by the buyer for the product.

Buyer´s exclusive remedy and BQC´s sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to BQC within 30 days of shipment.

Expiration date: 1 year from the date of fabrication. Expiration date is indicated on the outside of the box.

For further details, please refer to our website **bioquochem.com**

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REDOX TECHNOLOGIES

Vivero Ciencias de la Salud

C. Colegio Santo Domingo de Guzmán

33011 Oviedo, Asturias, Spain

www.bioquochem.com