NADP-Isocitrate Dehydrogenase (NADP-IDH) Assay Kit

Catalog No.: abx294420

Size: 96 tests

Detection Range: 0.10 U/L – 50.47 U/L

Sensitivity: 0.10 U/L

Storage: Store all components at -20°C in the dark.

Application: For detection and quantification of NADP-Isocitrate Dehydrogenase activity in tissue homogenates and cell lysates.

Introduction

Abbexa's NADP-Isocitrate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating NADP-Isocitrate Dehydrogenase activity. Isocitrate is converted to α-ketoglutarate, while $NADP^+$ is concurrently reduced to NADPH. The final reaction product has an absorbance maximum at 450 nm. NADP-Isocitrate Dehydrogenase activity can then be calculated by the change in absorbance values over time.

Kit components

1. 96-well microplate
2. Assay Buffer: 2 × 50 ml
3. Substrate: 1.5 ml
4. Accelerant Solution: 1.5 ml
5. Chromogenic Reagent: 2.5 ml
6. Standard (10 mmol/L): 0.5 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash in cold PBS (0.01 M, pH 7.4). Homogenize mechanically in 180 µl of Assay Buffer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then collect the supernatant and store on ice for immediate assay.
- **Cell Lysates:** Harvest 1 × 10^6 cells and wash with PBS (0.01 M, pH 7.4). Homogenize by ultrasonication in 200 µl of Assay Buffer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C, then collect the supernatant and store on ice for immediate assay.

  Note: To calculate NADP-Isocitrate Dehydrogenase activity in tissue homogenates or cell lysates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately **(abx097193)**.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Assay Buffer, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10 % Rat kidney tissue homogenate	10 – 60
10 % Rat brain tissue homogenate	5 – 10
10 % Rat liver tissue homogenate	10 – 80
CHO cell lysate	1
RAW cell lysate	1
293T cell lysate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Reaction Working Solution:** For each well to be assayed, mix 102 µl of Assay Buffer, 9 µl of Substrate, and 9 µl of Accelerant Solution. Store prepared solution on ice in the dark. Prepare fresh before use and use within 24 hours.
- **0.5 mmol/L Standard Solution:** Dilute 50 µl of Standard (10 mmol/L) with 950 µl of double distilled water and mix thoroughly. Store prepared solution on ice in the dark. Prepare fresh before use and use within 8 hours.
- **Standards:** Label 7 tubes with 0.1 mmol/L, 0.15 mmol/L, 0.2 mmol/L, 0.3 mmol/L, 0.35 mmol/L, 0.4 mmol/L, and 0.5 mmol/L. Prepare standard dilutions as summarized in the following table:

Standard Dilution (mmol/L)	**0.1**	**0.15**	**0.2**	**0.3**	**0.35**	**0.4**	**0.5**
0.5 mmol/L Standard (µl)	40	60	80	120	140	160	200
Double-distilled water (µl)	160	140	120	80	60	40	0

For the blank, or 0 mmol/L standard, use pure double-distilled water. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

1. Assign microplate wells for each standard and sample. *It is strongly recommended to prepare all the wells in duplicate.*
2. Add 20 µl of sample to sample wells, add 20 µl of the diluted standards to standard wells.
3. Add 120 µl of Reaction Working Solution to all wells.
4. Add 20 µl of Chromogenic Reagent to all wells.
5. Tap or shake the plate for 3 seconds to mix fully, then incubate at 37°C for 5 minutes in the dark.
6. Measure the OD of each well with a microplate reader at 450 nm, record the values as A_1.
7. Incubate at 37°C for 20 minutes in the dark.
8. Measure the OD of each well with a microplate reader at 450 nm, record the values as A_2.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions at A_2 (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of NADP-Isocitrate Dehydrogenase in each sample well can be derived with the following formula:

1. Tissue homogenate and Cell Lysate samples:

Total Protein

One unit of NADP-Isocitrate Dehydrogenase activity is defined as the amount required for 1 g of tissue/cell protein to produce 1 µmol of NADPH per minute at 37°C.

$$\text{NADP-IDH activity (U/g protein)} = \frac{(\Delta A_{450} - b)}{a \times T \times C_{Protein}} \times 1000 \times f$$

where:

ΔA_{450}	$\Delta A_{Sample} - \Delta A_{Blank}$
ΔA_{Sample}	Sample ODs $A_2 - A_1$
ΔA_{Blank}	0 mmol/L Standard ODs $A_2 - A_1$
T	Reaction time (20 minutes)
$C_{Protein}$	Concentration of protein in sample (g protein/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
1000	Conversion from mmol/L to µmol/L
f	The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.