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Isocitrate Dehydrogenase Assay Kit

Catalog No.: abx298898

Size: 96 tests

Storage: Store all reagents except Buffer Solution in the dark at -20°C for up to 12 months.

Application: For detection and quantification of isocitrate dehydrogenase (IDH) activity in cell lysates and tissue homogenates.

Introduction

Abbexa's Isocitrate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating IDH activity. IDH converts isocitrate to α-ketoglutaric acid, while concurrently NAD+ is reduced to NADH. NADH will then reduce WST-8 to form a yellow product with an absorbance maximum at 450 nm. The activity of IDH is proportional to the change in absorbance value at 450 nm.

Kit components

1. 96-well microplate
2. Buffer solution: 2 x 60 ml
3. Substrate: 1.6 ml
4. Accelerator: 2 vials
5. Chromogenic Reagent: 3 ml
6. Standard: 2 vials
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (450 nm)
2. Double distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Centrifuge
6. Incubator

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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:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled Buffer Solution. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately using abx097193.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT. 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 normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure to determine the optimal dilution of samples.

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

- **Accelerator Working Solution:** Mix one vial of accelerator with 1 ml of double distilled water, and mix thoroughly to dissolve. Aliquots can be stored at - 20°C in the dark for up to 5 days.
- **Reaction Working Solution:** For each well, prepare 120 µl of Reaction Working Solution (93 µl Buffer Solution, 9 µl Substrate, and 18 µl Accelerator Working Solution. Prepare immediately before carrying out the assay, and keep on ice in the dark throughout.
- **1 mmol/L Standard Solution:** Dissolve a vial of Standard with 1.6 ml of double distilled water and mix thoroughly. Prepare immediately before carrying out the assay.
- **Standard Curve:** Always prepare a fresh set of standards. Discard working solutions after use. Prepare standard curve as follows (the 0 mmol/L standard is the blank).

Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1**
1 mmol/L standard (µl)	0	40	60	80	120	160	180	200
Double distilled water (µl)	200	160	140	120	80	40	20	0

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B. Assay Procedure

1. Set control, standard and sample wells. Add 10 µl of 1 mmol/L Standard Solution to the relevant standard wells and 10 µl of sample to the sample wells and control wells. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
2. Add 120 µl of Reaction Working Solution to the standard and sample wells.
3. Add 120 µl of Buffer Solution to the control wells.
4. Add 20 µl of Chromogenic Reagent to each well.
5. Gently tap the plate to mix, or use a microplate shaker. Incubate at room temperature in the dark for 5 minutes.
6. Measure the OD value of each well with a microplate reader at 450 nm – this value is 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 – this value is A_2.

B. Calculation of Results

1. Standard curve:

Plot the standard curve, using the mean OD value of the standard dilutions (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 IDH activity in each sample well can be derived with the following formulae.

2. Tissues and cell lysate samples:

One unit of IDH activity is defined as the amount of IDH in 1 g of tissue protein that can produce 1 µmol of NADH at 37°C in 1 minute.

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

$\Delta A_{450} = \Delta A_{sample}$ - $\Delta A_{control}$

$\Delta A = \Delta A_2$ - ΔA_1

T = time of reaction, 20 min

C_p = concentration of protein in sample, g/L

f = dilution factor of sample before test

D. Technical Support

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

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