Isocitrate Dehydrogenase (IDH) Assay Kit (Colorimetric)

LS-K283-100 (100 Tests) • **Store at -20°C**

Introduction

ISOCITRATE DEHYDROGENASE (IDH) is an enzyme which catalyzes the interconversion of isocitrate and α-ketoglutarate. There are three IDH isoforms: IDH3 uses the cofactor NAD^+ and catalyzes the third step in the citric acid cycle, while IDH1 and IDH2 use the cofactor $NADP^+$ and catalyze the same reaction outside the citric acid cycle. This kit measures the activity of the $NADP^+$ isoforms. Mutations in IDH1 and IDH2 have been linked with various brain tumors and acute myeloid leukemia. This non-radioactive, colorimetric IDH assay is based on the reduction of the tetrazolium salt MTT in a NADPH-coupled enzymatic reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to the enzyme activity.

Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 0.1 to 100 U/L for 30 min reaction.
- Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

Applications

- IDH activity determination in biological samples (e.g. plasma, serum, tissue and culture media.)

Components

Component	K283-100
	100 Tests
Assay Buffer	10 mL
NADP/MTT	1 mL
Substrate	1 mL
Diaphorase	120 µL
Calibrator	1.5 mL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.

Storage

The kit is shipped at room temperature. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

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Assay Procedure

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. For optimal IDH activity the assay should be run at 37°C. The assay can be run at room temperature, but the activity of IDH is significantly reduced and a reaction time of >1 hour might be necessary for sufficient sensitivity.

Sample Preparation

Serum and plasma can be assayed directly.

Tissue: Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation

Keep thawed diaphorase on ice and equilibrate all other reagents to 37°C. Briefly centrifuge tubes before use.

Prepare enough Working Reagent (WR) for all assay wells by mixing, for each well, 9 µL Substrate, 9 µL NADP/MTT Solution, 1 µL Diaphorase and 70 µL Assay Buffer.

Fresh reconstitution of the WR is recommended.

Procedure

1. Transfer 100 µL H_2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into wells of a clear flat bottom 96-well plate.
2. Transfer 20 µL of each sample into separate wells. Add 80 µL WR to each sample well. Tap plate briefly to mix.
3. Incubate at 37°C. Use a plate reader to read OD_{565nm} at 10 minutes (OD_{10}), and again at 30 minutes (OD_{30}).

Calculations

Subtract the OD_{10} from OD_{30} for each sample to compute the ΔOD_S values. IDH activity can then be calculated as follows:

$$\text{IDH Activity} = \frac{\Delta OD_S}{\varepsilon_{mtt} \cdot l} \times \frac{\text{Reaction Vol (µL)}}{t\text{ (min)} \cdot \text{Sample Vol (µL)}} \times n$$

$$= \frac{\Delta OD_S}{OD_{CAL} - OD_{H20}} \times \frac{273}{t\text{ (min)}} \times n \quad (U/L)$$

where ε_{mtt} is the molar absorption coefficient of reduced MTT. l is the light path length which is calculated from the calibrator. OD_{CAL} and OD_{H20} are OD_{565nm} (OD_{10}) values of the Calibrator and water. t is the reaction time (20 min). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. n is the dilution factor.

Unit definition: 1 Unit (U) of IDH will catalyze the conversion of 1 µmole of isocitrate to α-ketoglutarate per min at 37°C and pH 8.2.

Note: If sample IDH activity exceeds 100 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with IDH activity < 5 U/L, the incubation time can be extended up to 2 hours for greater sensitivity.

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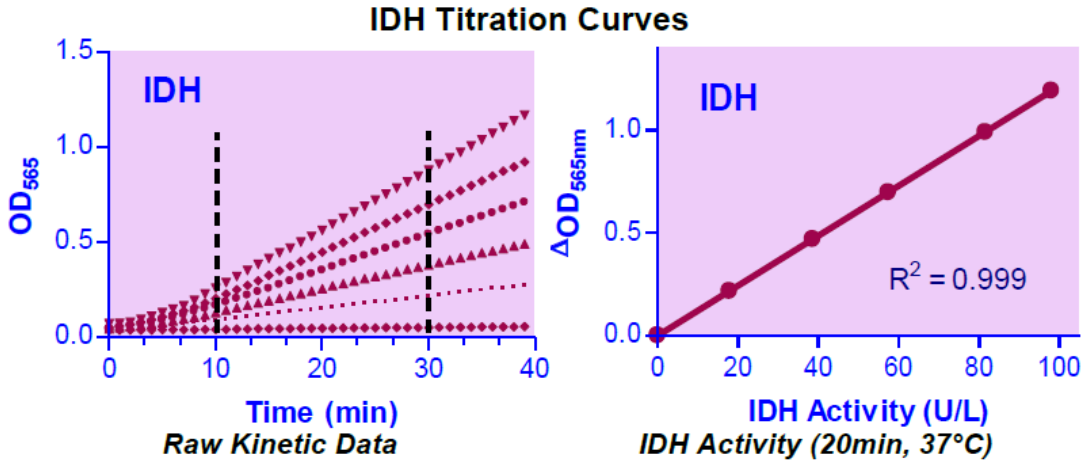
Sample Data



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