QuantiChrom™ Isocitrate Dehydrogenase Assay Kit (DIDH-100)

Quantitative Colorimetric Kinetic Isocitrate Dehydrogenase Activity Determination

DESCRIPTION

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. BioAssay Systems' 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.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Diaphorase:**	120 µL
NADP/MTT:	1 mL	**Calibrator:**	1.5 mL
Substrate:	1 mL		

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

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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.

Reaction Preparation:

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}).

CALCULATION

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

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

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

where ε_{mtt} is the molar absorption coefficient of reduced MTT. l is the light pathlength 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.

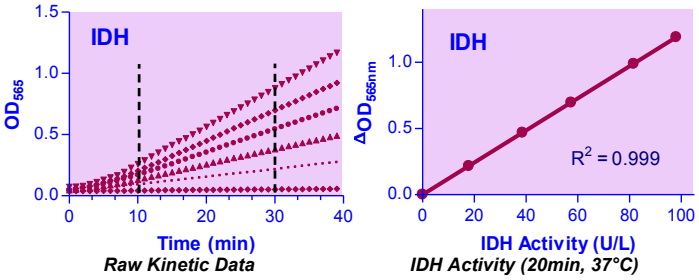
Unit definition: 1 Unit (U) of IDH will catalyze the conversion of 1 µmole of isocitrate to α-ketoglutarate per min at 37°C and pH 8.2.

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

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.

IDH Titration Curves



LITERATURE

1. Zhao, X et. al. (2013) Enzymatic Characterization of a Type II Isocitrate Dehydrogenase from Pathogenic Leptospira interrogans serovar Lai Strain 56601. Appl Biochem Biotechnol. Epub ahead of print.
2. Chen, C et. al. (2013) Cancer-associated IDH2 mutants drive an acute myeloid leukemia that is susceptible to Brd4 inhibition. Genes Dev. 27(18):1974-85.
3. Chaumeil, MM et. al. (2013) Non-invasive in vivo assessment of IDH1 mutational status in glioma. Nat Commun. 4:2429.