Technical Bulletin

Isocitrate Dehydrogenase Assay Kit

Catalogue number MAK493

Product 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.

The 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.

The linear detection range of the kit is 0.1-100 U/L. The kit is suitable for isocitrate dehydrogenase activity determination in biological samples such as plasma, serum, tissue, and culture media.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK493A
- Diaphorase 120 µL
  Catalogue Number MAK493B
- NADP/MTT 1 mL
  Catalogue Number MAK493C
- Substrate 1 mL
  Catalogue Number MAK493D
- Calibrator 1.5 mL
  Catalogue Number MAK493E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended
- Dounce tissue grinder set
  (Catalogue Number D9063 or equivalent)
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS)
  (Catalogue Number P3813 or equivalent)
- Potassium phosphate monobasic (Catalogue Number P0662 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at-20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.
Diaphorase: Keep thawed diaphorase on ice.
Equilibrate all other components to 37 °C.

Procedure

All Samples and calibrator should be run in duplicate.

Sample Preparation

Serum and plasma are assayed directly.

Tissue

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

Cell Lysate

1. Collect cells by centrifugation at 2,000 ×g for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5).
4. Centrifuge at 10,000 ×g for 15 minutes at 4 °C. Use the clear supernatant for the assay.

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

Working Reagent

Note: Fresh reconstitution of the Working Reagent is recommended.
For each well, prepare 89 µL of Working Reagent according to Table 1.

Table 1.

Preparation of Working Reagent

Reagent	Volume
Substrate	9 µL
NADP/MTT Solution	9 µL
Diaphorase	1 µL
Assay Buffer	70 µL

Assay Reaction

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to wells should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Note: 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.

1. Set the plate reader for the desired assay temperature of 37 °C.
2. Transfer 100 µL of purified water (OD_{H2O}) and 100 µL of Calibrator (OD_{CAL}) solution into separate wells of a clear flat bottom 96-well plate.
3. Transfer 20 µL of each Sample into separate wells of the plate.
4. Add 80 µL of Working Reagent to all Sample wells (not including the purified water (OH_{H2O}) and Calibrator (OD_{CAL}) wells). Tap plate lightly to mix.

Measurement

Incubate the plate at 37 °C. Read the optical density (OD) of each well at 565 nm at 10 min and again at 30 minutes (OD_{10Min} and OD_{30Min} respectively). Alternatively, using the plate reader's kinetic mode, monitor the OD for 30 minutes at 37 °C.

Results

1. Calculate the ΔOD_S values by subtracting the OD_{10} from OD_{30} for each sample.
2. IDH activity can then be calculated as follows:

IDH Activity (U/L) =

$$\frac{\Delta OD_S}{\varepsilon_{MTT} \times l} \times \frac{\text{Reaction Vol (µL)}}{t(\text{min}) \times \text{Sample Vol (µL)}} \times DF$$

$$= \frac{\Delta OD_S}{OD_{CAL} - OD_{H2O}} \times \frac{273}{t(\text{min})} \times DF \text{ (U/L)}$$

where:

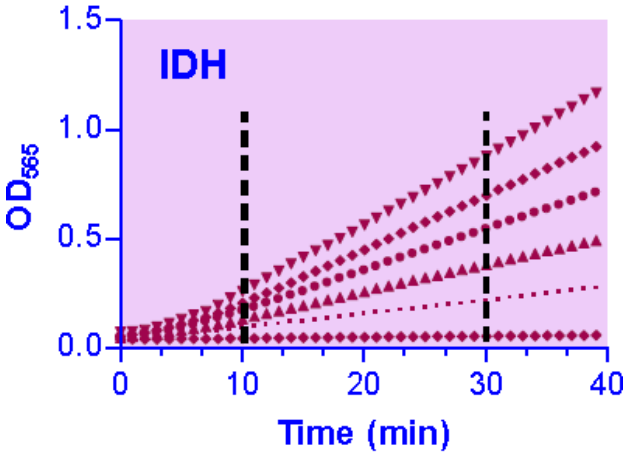
ε_{mtt} = molar absorption coefficient of reduced MTT.
l = light pathlength which is calculated from the calibrator.
OD_{CAL} = OD_{565nm} of the Calibrator
OD_{H20} = OD_{565nm} of the water
t = reaction time (20 minutes).
Reaction Vol = 100 µL
Sample Vol = 20 µL
DF = dilution factor

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.

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.

Figure 1

Typical Raw Kinetics Data



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