Technical Bulletin

Isocitrate Dehydrogenase Activity Assay Kit

Catalog Number MAK062

Product Description

Isocitrate dehydrogenase (IDH) catalyzes the conversion of isocitrate to α-ketoglutarate. In eukaryotes, there are three isozymes of IDH, the mitochondrial IDH2 and IDH3, and the cytoplasmic/ peroxisomal IDH1. All three IDH family members require the presence of a divalent cation (Mg^{2+} or Mn^{2+}) and either the electron-accepting cofactor $NADP^+$ (IDH1 and IDH2) or NAD^+ (IDH3) for their enzymatic activity. IDH1 and IDH2 mutations resulting in neomorphic enzymatic activity are found in certain cancers such as glioblastoma, acute myeloid leukemia, and colon cancer. This neoactivity shows a change in the substrate specificity resulting in the conversion of α-ketoglutarate to 2-hydroxyglutarate. Mutations in IDH family members are also associated with Ollier disease and Maffucci syndrome.

The Isocitrate Dehydrogenase Activity Assay Kit provides a simple and direct procedure for measuring $NADP^+$-dependent, NAD^+-dependent, or both $NADP^+$ and NAD^+-dependent IDH activity in a variety of samples. IDH activity is determined using isocitrate as the substrate in an enzyme reaction, which results in a colorimetric (450 nm) product proportional to the enzymatic activity present. One unit of IDH is the amount of enzyme that will generate 1.0 µmole of NADH or NADP per minute at pH 8.0 at 37 °C.

The kit is suitable for the measurement of isocitrate dehydrogenase (IDH) (NAD^+-dependent, $NADP^+$-dependent or both IDHs) activity in biological samples including tissue, cells, and serum.

Components

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

- IDH Assay Buffer — 25 mL
  Catalog Number MAK062A
- NAD^+ — 1 vl
  Catalog Number MAK062B
- $NADP^+$ — 1 vl
  Catalog Number MAK062C
- IDH Substrate — 1 vl
  Catalog Number MAK062D
- Developer — 1 vl
  Catalog Number MAK062E
- IDH Positive Control — 20 µL
  Catalog Number MAK062F
- NADH Standard, 0.5 µmole — 1 vl
  Catalog Number MAK062G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., 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 (Catalog Number D9063 or equivalent)
- Microcentrifuge capable of RCF ≥ 13,000 × *g*

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, protected from light.

Preparation Instructions

Briefly centrifuge vials prior to opening.

IDH Assay Buffer - Allow buffer to come to room temperature before use.

NAD^+, $NADP^+$, and IDH Substrate - Reconstitute each vial with 220 µL of purified water. Mix well by pipetting, then aliquot and store protected from light at –20 °C. Use within 2 months of reconstitution. Avoid repeated freeze/thaw cycles.

Developer - Reconstitute with 0.9 mL of purified water. Mix well by pipetting (**do NOT vortex**), then aliquot and store protected from light at –20 °C. Use within 2 months of reconstitution. Avoid repeated freeze/thaw cycles.

NADH Standard - Reconstitute vial with 50 µL of purified water to generate a 10 mM NADH stock solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution. Avoid repeated freeze/thaw cycles.

Procedure

All samples should be run in duplicate.

Sample Preparation

Tissue (50 mg) or cells (1×10^6) can be homogenized in 200 µL of ice-cold IDH Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum samples can be directly added to wells.

Prepare two parallel Sample wells, with one well serving as a Sample Background Control. Add 5-50 µL of Sample to both wells. For unknown samples, perform a pilot experiment by testing several amounts to ensure the readings are within the range of the Standard Curve. Bring Sample and Sample Background Control wells to a final volume of 50 µL with IDH Assay Buffer.

For the Positive Control (optional), add 2-5 µL of the IDH Positive Control solution to wells and adjust to 50 µL with the IDH Assay Buffer.

Standard Curve Preparation

1. A new standard curve must be set up each time the assay is run.
2. Prepare a 1 mM NADH Standard by mixing 10 µL of the 10 mM NADH Standard with 90 µL of IDH Assay Buffer.
3. Prepare NADH standards according to Table 1. The NADH standard curve can be used as the standard for the NAD^+-dependent IDH as well as the $NADP^+$-dependent IDH activity.

Table 1.
Preparation of NADH Standards

Well	1 mM NADH Standard	IDH Assay Buffer	NADH (nmole/ well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Reaction Mix

1. Mix enough reagent for the number of assays to be performed. For each well, prepare 50 µL of appropriate Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix	Background Control Mix
IDH Assay Buffer	38 µL	42 µL
Developer	8 µL	8 µL
IDH Substrate	2 µL	-
NAD^+ and/or $NADP^+$ (see Note)	2 µL	-

Note: For Test Samples, if 2 µL NAD^+ is added, the assay will detect NAD^+-dependent IDH. If 2 µL $NADP^+$ is added, the assay will detect $NADP^+$-dependent IDH. The addition of both 2 µL NAD^+ and 2 µL $NADP^+$ will detect total IDH activity. If both NAD^+ and $NADP^+$ are added to the Reaction Mix, adjust the volume of IDH Assay Buffer to 36 µL.

2. Add 50 µL of the Reaction Mix to each Test Sample, Positive Control, and Standard well. Add 50 µL of the Background Control Mix to each Background Control well. Mix well.

Measurement

1. Incubate the plate for 3 minutes at 37 °C.
2. Measure the optical density (OD) at 450 nm in a microplate reader.
3. It is recommended to measure the OD in kinetic mode (every 1 – 5 minutes). Incubate the plate for an additional 30 minutes to 2 hours at 37 °C. Incubation time will depend on the IDH activity in the Test Samples.
4. The NADH Standard Curve can be read (OD_{END}) at 450 nm in Endpoint Mode (i.e., at the end of the incubation time).

Results

1. Subtract the OD_{END} 0 Standard value from all OD_{END} Standard readings.
2. Plot the NADH Standard Curve.
3. Choose two time points (T_2 and T_1) in the linear range to calculate the IDH activity of the Samples.
4. Calculate the change in optical density for the Test samples: $\Delta OD = A_2 - A_1$.
5. If the Sample Background Control reading is significant, subtract the Sample Background Control ΔOD reading from the Test Sample ΔOD reading. If the Sample Background Control reading is insignificant, subtract the 0 Standard value from the Test Sample ΔOD reading.
6. Apply the corrected Test Sample ΔOD to the NADH standard curve to get B nmol of NAD(P)H generated by IDH during the reaction time ($\Delta T = T_2 - T_1$).

IDH (nmoles/min/mL or mU/mL) =

$$\frac{B}{\Delta T - V} \; x \; DF$$

where:

B = NAD(P)H amount from the Standard Curve (in nmol).

ΔT = Reaction time (in min) ($\Delta T = T_2 - T_1$).

V = Sample volume added into the reaction well (in mL)

DF = Sample dilution factor

Unit Definition: One unit of IDH is the amount of enzyme that will generate 1.0 µmol of NADH or NADPH per minute at pH 8 at 37 °C.

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