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Product Information

Isocitrate Dehydrogenase Activity Assay Kit

Catalog Number **MAK062**
Storage Temperature –20 °C

TECHNICAL BULLETIN

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.

Components

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

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

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

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

NAD^+, $NADP^+$, and IDH Substrate Buffer – Reconstitute each with 220 μL of water. Mix well by pipetting, then aliquot and store, protected from light at –20 °C. Use within 2 months of reconstitution.

Developer – Reconstitute with 0.9 mL of water. Mix well by pipetting (don't vortex), then aliquot and store, protected from light at –20 °C. Use within 2 months of reconstitution.

NADH Standard – Reconstitute in 50 μL of 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.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

NADH Standards for Colorimetric Detection
Dilute 10 µL of the 10 mM NADH Standard with 90 µL of the IDH Assay Buffer to prepare a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add IDH Assay Buffer to each well to bring the volume to 50 µL. The NADH standard curve can be used as the standard for the NAD^+-dependent IDH as well as the $NADP^+$-dependent IDH activity.

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.

Bring samples to a final volume of 50 µL with IDH Assay Buffer.
Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For the positive control (optional), add 2 µL of the IDH positive control solution to wells and adjust to 50 µL with the IDH Assay Buffer.

Assay Reaction
1. Set up the Master Reaction Mixes according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).
   Note: NADPH and NADH in the samples will generate a background signal. To remove the effect of NADPH and NADH background, a sample blank may be set up for each sample by omitting the IDH substrate and NAD^+ and/or $NADP^+$.

Table 1.
Master Reaction Mixes

Reagent	Standards and Samples	Sample Blank
IDH Assay Buffer	38 µL	42 µL
Developer	8 µL	8 µL
IDH Substrate	2 µL	–
NAD^+ and/or $NADP^+$	2 µL	–

Note: For 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, adjust the volume of IDH Assay Buffer to 36 µL.

2. Add 50 µL of the appropriate Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at 37 °C. After 2–3 minutes ($T_{initial}$), measure the absorbance at 450 nm $(A_{450})_{initial}$.
   Note: It is essential that $(A_{450})_{initial}$ is in the linear range of the standard curve.

4. Continue to incubate the plate at 37 °C measuring the absorbance (A_{450}) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (10 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final absorbance measurement $[(A_{450})_{final}]$ for calculating the enzyme activity would be the penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve (see step 5). The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Results

Calculations

Correct for the background by subtracting the final measurement [$(A_{450})_{final}$] obtained for the 0 (blank) NADH standard from the final measurement [$(A_{450})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the NADH standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in absorbance from $T_{initial}$ to T_{final} for the samples.

$$\Delta A_{450} = (A_{450})_{final} - (A_{450})_{initial}$$

Compare the ΔA_{450} of each sample to the standard curve to determine the amount of NADH and/or NADPH (B) generated by the isocitrate dehydrogenase between $T_{initial}$ and T_{final}.

The IDH activity of a sample may be determined by the following equation:

$$\text{IDH Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of NADH generated between $T_{initial}$ and T_{final}.

Reaction Time = $T_{final} - T_{initial}$ (minutes)

V = sample volume (mL) added to well

IDH activity is reported as nmole/min/mL = milliunit/mL. 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.

Example:

NADH (B) = 5.84 nmole

First reading ($T_{initial}$) = 3 minute

Second reading (T_{final}) = 32 minutes

Sample volume (V) = 0.01 mL

Sample dilution is 1

IDH activity is:

$$\frac{5.84 \times 1}{(32-3) \times 0.01} = 20.14 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until used
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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