Recombinant Human Isocitrate Dehydrogenase 1/IDH1

Catalog Number: 7049-DH

DESCRIPTION

Source	*E. coli*-derived human Isocitrate Dehydrogenase 1/IDH1 protein Met1-Leu414, with a C-terminal 6-His tag Accession # O75874
N-terminal Sequence Analysis	Ser2
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

SDS-PAGE	43-45 kDa, reducing conditions.
Activity	Measured by the ability to oxidatively decarboxylate isocitrate to 2-oxoglutarate. The specific activity is >10,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol, Brij-35 and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 0.5 mM $MnCl_2$, 5 mM DTT, pH 7.5
- Recombinant Human Isocitrate Dehydrogenase 1 (rhIDH1) (Catalog # 7049-DH)
- DL-Isocitric acid (Sigma, Catalog # I1252), 100 mM stock in deionized water
- $NADP^+$ (Sigma, Catalog # N5755), 50 mM stock in deionized water
- 96-well Clear Plate (Costar, Catalog # 2592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhIDH1 to 0.4 ng/µL in Assay Buffer.
2. Prepare a Substrate Mixture by Diluting $NADP^+$ and Isocitric Acid to 1 mM and 2 mM, respectively, in Assay Buffer.
3. Load into a plate 50 µL of 0.4 ng/µL rhIDH1 and start the reaction by adding 50 µL of Substrate Mix. For Substrate Blanks, load 50 µL of Assay Buffer and 50 µL of Substrate Mix.
4. Read plate at a wavelength of 340 nm (bottom read) in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 6270 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhIDH1: 0.020 µg
- $NADP^+$: 0.5 mM
- Isocitric Acid: 1 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Isocitrate Dehydrogenase 1 (IDH1) catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate. There are two subclasses in the IDH family, one of them utilizing $NADP^+$ as the electron acceptor and the other using NAD^+ (1). The protein encoded by this gene is the $NADP^+$-dependent isocitrate dehydrogenase found in the cytoplasm and peroxisomes. In peroxisomes, IDH1 generates the NADPH required for intraperoxisomal reduction reactions. Mutations of Arg132 of human IDH1 result in a reduced ability of the enzyme to convert isocitrate to α-ketoglutarate, but the enzyme acquires the ability to generate 2-hydroxyglutarate (2HG) from α-ketoglutarate (2). Elevated levels of the metabolite 2HG are associated with a high risk of malignant brain tumors. Arg132 mutations of IDH1 are common in high-grade gliomas, but not in other types of tumors (3).

References:

1. Nekrutenko, A. *et al.* (1998) Mol. Biol. Evol. **15**:1674.
2. Dang, L. *et al.* (2009) Nature **462**:739.
3. Bleeker, F.E. *et al.* (2009) Hum. Mutat. **30**:7.

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