Recombinant Human DHODH His-tag

Catalog Number: 10062-DD

DESCRIPTION

Source	*E. coli*-derived human DHODH protein Thr31 - Arg395 Accession # Q02127 with N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	41 kDa

SPECIFICATIONS

SDS-PAGE	41 kDa, reducing conditions
Activity	Measured by its reduction of 2,6-dichloroindophenol during the oxidation of dihydroorotate. The specific activity is >15000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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 and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 150 mM KCl, 0.1% Triton® X-100, pH 8.0
- Recombinant Human DHODH His-tag (rhDHODH) (Catalog # 10062-DD)
- L-Dihydroorotic acid (Sigma, Catalog # D7128), 40 mM stock in DMF
- Decylubiquinone (Sigma, Catalog # D7911), 20 mM stock in DMSO
- 2,6-Dichloroindophenol sodium salt hydrate (DPIP) (Sigma, Catalog # D1878), 2 mM in Absolute Ethanol
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhDHODH to 0.4 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 2 mM L-Dihydroorotic acid, 0.2 mM Decylubiquinone and 0.12 mM DPIP in Assay Buffer.
3. Load 50 µL of 0.4 µg/mL rhDHODH to plate, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of Substrate Mixture.
4. Immediately read plate in kinetic mode for 5 minutes at 600 nm (absorbance).
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Using the extinction coefficient 21,000 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:

- rhDHODH: 0.02 µg
- L-Dihydroorotic acid: 1 mM
- Decylubiquinone: 0.1 mM
- DPIP: 0.06 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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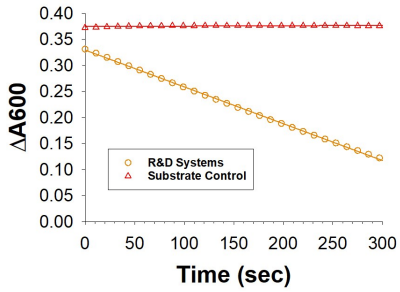
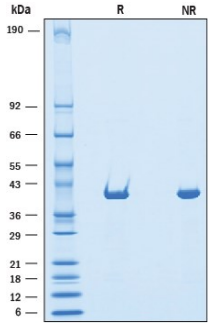
Binding Activity



Recombinant Human DHODH (Catalog # 10062-DD) is measured by its reduction of 2,6-dichloroindophenol during oxidation of dihydroorotate.

SDS-PAGE



2 µg/lane of Recombinant Human DHODH was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at 41 kDa.

BACKGROUND

Dihydroorotate dehydrogenase (DHODH) is a rate-limiting enzyme in the pyrimidine de novo pathway that converts dihydroorotate to orotate. DHODHs have a monomeric structure composed of a large C-terminal a/b barrel and a small N-terminal helical domain (1). There are two main classes of DHODHs based on similarity, preference of substrate, and subcellular location (1,2). Within Class 2, structural differences can be exploited for selective targeting (3,4). Human DHODH belongs to Class 2 and is a monomeric, mitochondrial, FMN-dependent enzyme (2). It is ubiquitously expressed in most tissue. As DHODH catalyzes de novo pyrimidine synthesis for synthesis of DNA/RNA essential to rapidly proliferating cells, DHODH is currently a target for treatment of cancer (5-8). It has also been successfully targeted in treatment for rheumatoid arthritis (9-10), multiple sclerosis (11-12), viral infection (13-14), microbial infectious diseases such as malaria (4, 15) and gastrointestinal disease (3, 16), and antifungal infection (17). Mutations in the DHODH resulting in functional defects cause Miller syndrome, also known as postaxial acrofacial dystosis syndrome (POADS) (18).

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