Recombinant Human Dihydrolipoamide Dehydrogenase/DLD  
Catalog Number: 8646-DH

DESCRIPTION

Source  E. coli-derived

<table>
<thead>
<tr>
<th>N-terminal</th>
<th>C-terminal</th>
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<tbody>
<tr>
<td>GGS</td>
<td>HHHHHH</td>
</tr>
<tr>
<td>GMASLENLYFQ</td>
<td>Human DLD</td>
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<tr>
<td>(Ala36-Phe509)</td>
<td>Accession # P09622</td>
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</tbody>
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N-terminal Sequence  Gly Analysis

Predicted Molecular Mass  52 kDa

SPECIFICATIONS

SDS-PAGE  52-62 kDa, reducing conditions

Activity  Measured by its ability to produce NADH during the oxidation of lipoic acid.

The specific activity is >5,000 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 under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

Formulation  Supplied as a 0.2 μm filtered solution in Sodium Phosphate and Sucrose. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Sodium Phosphate, 1 mM EDTA, 1 mg/mL BSA, pH 5.5
- Recombinant Human Dihydrolipoamide Dehydrogenase/DLD (rhDLD) (Catalog # 8646-DH)
- NADH (Sigma, Catalog # N8129), 20 mM stock in 0.1 M Sodium Borate, pH 9.0
- NAD (Sigma, Catalog # N6522), 100 mM stock in deionized water
- (±)-α-Lipoic acid (Sigma, Catalog # T1395), 20 mM stock in 95% Ethanol
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Create a Substrate Mixture containing 0.4 mM NADH, 0.2 mM NAD and 2 mM Lipoic Acid in Assay Buffer.
2. Incubate Substrate Mixture at room temperature for 5 minutes in the dark.
3. Dilute rhDLD to 2 μg/mL in Assay Buffer.
4. Load 50 μL of 2 μg/mL rhDLD in a 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.
5. Read plate in kinetic mode for 5 minutes at an absorbance of 340 nm.
6. Calculate specific activity:

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

   *Adjusted for Substrate Blank
   ** Using the extinction coefficient 6220 M⁻¹cm⁻¹
   *** Using the path correction 0.32 cm

   Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhDLD: 0.1 μg
- NADH: 0.2 mM
- NAD: 0.1 mM
- Lipoic Acid: 1 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.
Dihydrolipoamide dehydrogenase (DLD), also known as LADH, is an NAD-dependent oxidoreductase in the mitochondrial matrix (1). DLD serves as the E3 subunit of four mitochondrial enzyme complexes: pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, branched chain alpha-ketotacid dehydrogenase, and the glycine cleavage system (2, 3). It is active as a 120 kDa dimer that catalyzes oxidation within these enzyme complexes. Several mutations of human DLD have been described, some of which contribute to the loss of respiratory function during oxidative stress (4, 5). DLD mutations located at the interface between dimer subunits can impair dimer formation (5, 6). The involvement of DLD in the regulation of lipid peroxidation and lactate metabolism is important for mouse hippocampal neuroblast proliferation and hamster sperm capacitation, respectively (7, 8). DLD polymorphisms in insects can increase their resistance to the pesticide phosphine gas, while they can increase the sensitivity to arsenite in the nematode C. elegans (9). Mature human DLD shares 95-96% amino acid (aa) sequence identity with hamster, mouse, and rat DLD. Alternative splicing generates additional human DLD isoforms that lack the N-terminal 99 aa or carry a deletion of aa 147-194.

References: