

DESCRIPTION

Source *E. coli*-derived
Ala2-Leu334, with N-terminal Met and 6-His tag
Accession # P07195

N-terminal Sequence Analysis Met

Predicted Molecular Mass 37 kDa

SPECIFICATIONS

SDS-PAGE 36 kDa, reducing conditions

Activity Measured by its ability to reduce pyruvate to lactate.
The specific activity is >60,000 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, TCEP and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 25 mM Tris, 100 mM NaCl, pH 7.5
 - Recombinant Human LDHB (rhLDHB) (Catalog # 9205-HB)
 - β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate (β-NADH) (Sigma, Catalog # N8129), 20 mM stock in 0.1 M Sodium Borate, pH 9.0
 - 100 mM Sodium Pyruvate (Gibco, Catalog # 11360)
 - 96-well Clear Plate (Catalog # DY990)
 - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
1. Dilute rhLDHB to 0.4 ng/μL in Assay Buffer.
 2. Prepare a substrate mixture containing 1.6 mM β-NADH and 4 mM sodium pyruvate in Assay Buffer.
 3. In a plate load 50 μL of 0.4 ng/μL rhLDHB, 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. Read plate at 340 nm (absorbance) in kinetic mode for 5 minutes.
 5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Using the extinction coefficient 6220 M⁻¹cm⁻¹

***Using the path correction 0.320 cm

Note: the output of many spectrophotometers is in mOD.

- Final Assay Conditions**
- Per Well:
- rhLDHB: 0.020 μg
 - β-NADH: 0.8 mM
 - Sodium pyruvate: 2 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.

BACKGROUND

A hallmark of most cancer cells is an altered metabolism involving a shift to aerobic glycolysis with lactate production coupled with a higher uptake of glucose as the main source of energy. Lactate dehydrogenase (LDH) is key to this shift by catalyzing the formation of lactate by reducing pyruvate with NADH, which also generates NAD(+) that is essential for the continuity of glycolysis (1). Inhibiting LDH activity has an anti-proliferative effect on cancer cells (2). It may reverse the resistance of tumor cells to conventional chemo- and radiotherapy. Recent research has renewed interest in LDH as an anticancer drug target (3). LDH enzymes have three homologous subunits LDHA, LDHB and LDHC. The combination of these three results to multiple isozymes, including LDH-M and LDH-H. Mutations in LDHB cause lactate dehydrogenase B deficiency, a condition with no deleterious effects on health (4).

References:

1. Faloppi L. *et al.* (2016) *Sci Rep.* **6**:24136.
2. Ghosh, M. *et al.* (2016) *Chem. Commun.* **52**:2401.
3. Augoff, K. *et al.* (2015) *Cancer Lett.* **358**:1.
4. Maekawa M. *et al.* (1993). *Hum. Genet.* **91**:423.
5. Maekawa M. *et al.* (1990). *Biochem. Biophys. Res. Commun.* **168**:677.