Recombinant Human Lactate Dehydrogenase A/LDHA

Catalog Number: 9158-HA

DESCRIPTION

Source	*E. coli*-derived Ala2-Phe332, with N-terminal Met and 6-His tag Accession # P00338
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	38 kDa

SPECIFICATIONS

SDS-PAGE	36 kDa, reducing conditions
Activity	Measured by its ability to reduce pyruvate to lactate. The specific activity is >95,000 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 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 Lactate Dehydrogenase A/LDHA (rhLDHA) (Catalog # 9158-HA)
- 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 rhLDHA 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 rhLDHA, 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:

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

*Adjusted for Substrate Blank.
**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$.
***Using the path correction 0.320 cm.
Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions

Per Well:

- rhLDHA: 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). LDHA is a key enzyme that controls the production of lactate in the glycolysis pathway. It is therefore an important control point in the system of cellular energy release. It's up regulation is common in many malignant tumors. 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).The protein is found predominantly in muscle tissue. Mutations in LDHA have been linked to exertional myoglobinuria (4).

References:

1. Faloppi L. *et al.* (2016) Sci Rep. doi: 10.1038/srep24136.
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.* (1990). Biochem. Biophys. Res. Commun. **168**:677.

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