Human Lactate Dehydrogenase A Inhibitor Screening Kit (Colorimetric)

(Catalog #NBP2-59751 Store at -20°C)

I. Introduction:

The human oxidoreductase Lactate Dehydrogenase A (LDHA) (EC:1.1.1.27) is a key enzyme in lactic acid metabolism. It interconverts pyruvate and NADH to lactate and NAD^+ respectively. In mammals, LDHA exists as a tetramer and is primarily found in skeletal muscle tissues. LDHA is a common marker to assay for tissue damage and is also known to promote tumor proliferation in breast, lung, and pancreatic cancer patients. In Novus' LDHA Inhibitor Screening Kit, LDHA catalyzes the conversion of lactate to an intermediate that interacts with a probe to develop a color that can be measured at OD 450 nm. In the presence of a LDHA specific inhibitor, the reaction is impeded. A LDHA Inhibitor Control is included to compare the efficacy of the sample inhibitors. The assay is high-throughput adaptable and can be completed in less than 1 hr.

$$\textbf{Lactate + NAD} \xrightarrow{\text{LDH + Inhibitor}} \text{Intermediates} \xrightarrow{\text{Probe}} \textbf{Color (}OD\textbf{=450 nm)}$$

II. Application:

- Screening/characterizing/studying potential inhibitors for LDHA.

III. Kit Contents:

Components	NBP2-59751	Cap Code
LDHA Assay Buffer	25 ml	WM
LDHA Substrate Mix	1 vial	Red
LDHA	15 µl	Green
LDHA Inhibitor Control	1 vial	Blue
LDHA Dilution buffer	1 ml	Clear

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **LDHA:** Ready to use. Store at -20°C.
- **LDHA Inhibitor Control:** Reconstitute with 50 µl LDHA Assay Buffer. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **LDHA Substrate Mix:** Dissolve with 220 µl ddH$_2$O.

VI. LDHA Inhibitor Screening Protocol:

1. **Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve candidate inhibitors into an appropriate solvent. Dilute to 2X desired test concentration with LDHA Assay Buffer. Add 50 µl diluted candidate inhibitor or LDHA Assay Buffer into desired wells for Sample Screen [S], and Enzyme Control [EC] (no inhibitor) respectively. For the Inhibitor Control (IC): add 2 µl of Inhibitor Control into desired well(s). Adjust the volume to 50 µl.

   Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. Prepare a solvent control [SC] to test the effect of solvent on the enzymatic activity.

2. **LDHA Enzyme Preparation:** Make enough diluted LDHA enzyme for the number of assays to be performed. Dilute LDHA 1:40 with assay buffer, e.g. dilute 2 µl of LDHA into 78 µl LDHA Dilution Buffer, mix well. Add 5 µl diluted LDHA Enzyme into Sample, Enzyme Control and Inhibitor Control wells and incubate the mixture for 10 minutes at 25 ℃.

   Note: * Discard the diluted LDHA after 24 hours.

3. **Substrate Solution Preparation:** Make enough reagents for the number of assays to be performed. For each well, prepare 40 µl of Substrate Solution containing:

LDHA Assay Buffer	43 µl
LDHA Substrate Mix	2 µl

   Mix and add 45 µl of Substrate solution into each well. Mix well with gentle shaking.

4. **Measurement:** Measure OD$_{450nm}$ in kinetic mode for 30 min at 25°C. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the OD$_{450nm}$ (OD$_1$ & OD$_2$).

5. **Calculations:** Calculate the slope for all sample including the Enzyme Control (EC), Inhibitor Control (IC), Sample (S), and Solvent Controsl (S). Consider EC as 100%, by dividing the net ΔOD (=OD$_2$-OD$_1$) value by the time Δt = (t_2-t_1). Calculate % relative inhibition as follows:
   Note: * If the activity measured in your solvent control (SC) is significantly different from that in your enzyme control (EC) use the SC value instead of EC value in the equations below.

$$\text{Relative Activity } (\%) = \frac{\text{Slope of S}}{\text{Slope of EC}} \text{ X } 100$$

$$\text{Relative Inhibition } (\%) = \frac{\text{Slope of EC} - \text{Slope of S}}{\text{Slope of EC}} \text{ X } 100$$

Where: **Slope of EC** is the slope of Enzyme Control
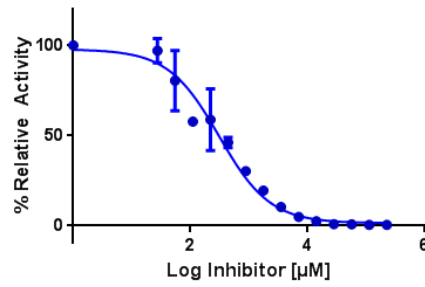**Slope of S** is the slope of Sample Screen

Figure: Inhibition of LDHA activity by LDHA Inhibitor (IC_{50} = 310 µM). Assay was performed following the kit protocol.

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