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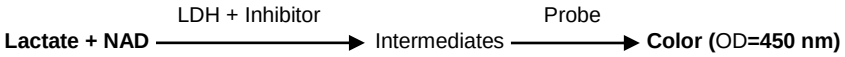
Human Lactate Dehydrogenase A Inhibitor Screening Kit (Colorimetric)

(Catalog # K492-100; 100 assays; 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 BioVision's 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.



II. Application:

- Screening/characterizing/studying potential inhibitors for LDHA.

III. Kit Contents:

Components	K492-100	Cap Code	Part Number
LDHA Assay Buffer	25 ml	WM	K492-100-1
LDHA Substrate Mix	1 vial	Red	K492-100-2
LDHA	15 µl	Green	K492-100-3
LDHA Inhibitor Control	1 vial	Blue	K492-100-4
LDHA Dilution buffer	1 ml	Clear	K492-100-5

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_2O.

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 °C.

   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.

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

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

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

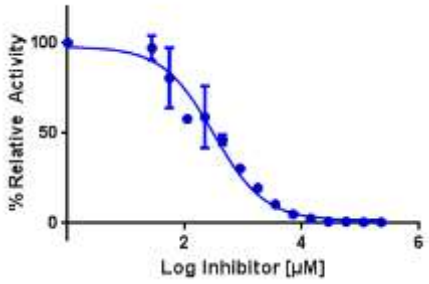


Figure: Inhibition of LDHA activity by LDHA Inhibitor (IC_{50} = 310 µM). Assay was performed following the kit protocol.

VII. RELATED PRODUCTS:

Lactate Dehydrogenase Activity Colorimetric Assay (K726)
Lactate Colorimetric/Fluorometric Assay Kit (K607)
Human Recombinant LDHA (6374)
Human Recombinant LDHB (6375)
EZScreen™ Lactate Dehydrogenase Activity Assay (K953)
PicoProbe™ Lactate Dehydrogenase Activity Assay (K730)
Sodium Oxamate (2580)
PicoProbe™ Lactate Fluorometric Assay Kit (K638)
D-Lactate Colorimetric Assay Kit (K667)
PicoProbe™ LDH-Cytotoxicity Fluorometric Assay (K311)
Enolase Activity Colorimetric/Fluorometric Assay (K691)
Human Recombinant Alpha-Enolase (6363)
IDH1 R132H Mutant Inhibitor Screening Kit (Colorimetric) (K493)
Mutant Isocitrate Dehydrogenase (Mutant IDH) Activity Assay (Colorimetric) (K985)

FOR RESEARCH USE ONLY! Not to be used on humans.