

Add 50 μ l of the Reaction Mix to each well containing the Standards, Positive Control, and test samples.

4. Measurement: Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10-30 min. at 37°C.

Note: Incubation time depends on the LDH activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the LDH activity of the samples. The NADH Standard Curve can be read in endpoint mode (i.e. at the end of incubation time).

5. Calculation: Subtract 0 Standard reading from all readings. Plot the NADH Standard curve. Calculate the LDH activity of the test sample: $\Delta\text{RFU} = A_2 - A_1$. Apply the ΔRFU to the NADH Standard Curve to get B pmol of NADH generated by LDH during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{Sample LDH Activity} = \frac{B}{(\Delta T \times V)} \times D = \text{pmol/min}/\mu\text{l} = \mu\text{U}/\mu\text{l} = \text{U/L}$$

Where: **B** is NADH amount from the Standard Curve (pmol)

ΔT is reaction time (min.)

V is sample volume added into the reaction well (μ l)

D is dilution factor

LDH activity in samples can be expressed in mU/mg of protein.

Unit Definition: One unit of Lactate Dehydrogenase is the amount of enzyme that generates 1.0 μ mol of NADH per min. at pH 8.8 at 37°C.

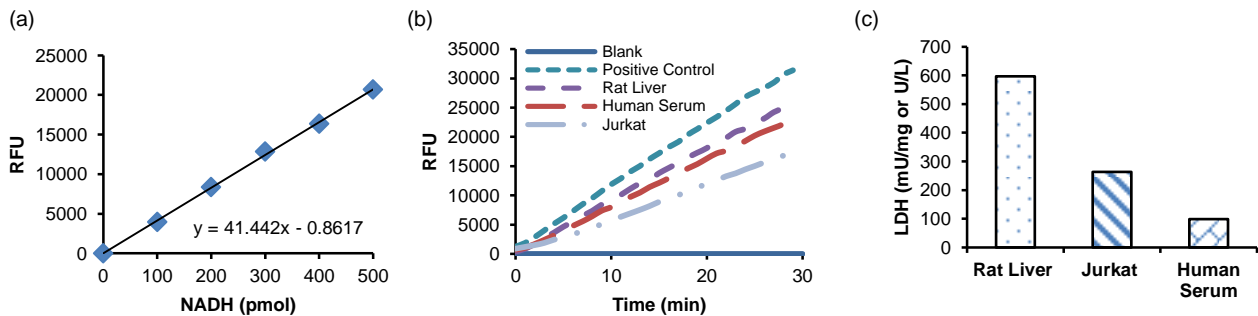


Figure: (a) NADH Standard Curve, (b) Kinetic measurement of Lactate Dehydrogenase activity in various samples and (c) Relative LDH Activity was calculated in lysates prepared from rat liver (0.037 μ g protein), Jurkat cells (0.053 μ g protein), and human serum (0.2 μ g protein). Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Lactate Dehydrogenase Activity Assay Kit (K726)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
Pyruvate Dehydrogenase Activity Assay Kit (K679)
Lactate Colorimetric Assay Kit II (K627)
Phosphoglucomutase Assay Kit (K774)
PicoProbe™ D-Lactate Fluorometric Assay Kit (K668)
Glucose-6-Phosphate Assay Kit (K657)
LDH Antibody (3842)
Sodium oxamate (2580)

LDH-Cytotoxicity Colorimetric Assay Kit (K311-400)
LDH-Cytotoxicity Colorimetric Assay Kit II (K313-500)
Lactate Colorimetric/Fluorometric Assay Kit (K607)
D-Lactate Colorimetric Assay Kit (K667)
Phosphoglucose Isomerase Assay Kit (K775)
Glucose-1-Phosphate Assay Kit (K697)
Glucose Dehydrogenase Activity Assay Kit (K786)
(\pm)-Gossypol (2579)

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