# CheKine™ Lactate Dehydrogenase Assay Kit

| Item NO. | Product Name |
| --- | --- |
| KTB1110 | CheKine™ Lactate Dehydrogenase Assay Kit |

**ATTENTION**

*For laboratory research use only. Not for clinical or diagnostic use*

**Version 201904**

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# INTRODUCTION

## Background

Lactate dehydrogenase (LDH) is an oxidoreductase (EC 1.1.1.27) presents in a wide variety of organisms. It catalyses the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD+. It converts pyruvate, the final product of glycolysis, to lactate in hypoxic conditions. LDH quantification is of clinical interest as serum levels of certain LDH isozymes reflect pathological conditions in particular tissues. When disease or injury or toxic material damages tissues, the cells LDH is released into the bloodstream. Since LDH is a fairly stable enzyme, LDH has been widely used to evaluate the presence of damage and toxicity of tissue and cells.

## Assay principle

CheKine™ Lactate Dehydrogenase Assay Kit provides a simple and easy colorimetric assay for measuring Lactate Dehydrogenase in serum, plasma, cell culture supernatants, tissue/cell lysates, fermentation and other biological fluids. In this colorimetric assay, LDH reduces NAD to NADH, which then interacts with a probe to produce a color (λmax = 450 nm). The assay is quick and convenient.

## Detection Range

The kit can detect 1- 20 U/mL of LDH directly in samples.

## Storage/Stability

Storage at –20°C and Keep from light. Stable for at least 6 months at recommended temperature from date of shipment.

## Assay Restrictions

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## PRODUCT INFORMATION

### Materials supplied and Storage conditions

| Kit components | Quantity | | | Storage conditions |
|---|---|---|---|---|
| | 48T | 96T | 480T | |
| Assay Buffer | 15 mL | 25 mL | 100 mL | 4ºC |
| Lactate | 0.6 mL | 1.2 mL | 6 mL | -20ºC |
| NAD | 0.5 mL | 1 mL | 5 mL | -20ºC |
| WST-8 | 300 μL | 600 μL | 3 mL | -20ºC, protect from light |
| Enhancer | 60 μL | 120 μL | 600 μL | -20ºC, protect from light |
| Lactate Dehydrogenase Standard (100 U/mL) | 0.25 mL | 0.5 mL | 2.5 mL | -20ºC |

### Other supplies required, Not Supplied

- Standard microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- Assorted glassware for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)

### Technical hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

## ASSAY PROTOCOL

### Reagent preparation

Note: Briefly centrifuge small vials at low speed prior to opening.

**Assay Buffer**: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4ºC.
**Lactate**: Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
**NAD**: Ready to use as supplied. Keep on ice protected from light during the assay. Aliquot standard so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
**WST-8**: Ready to use as supplied. Keep on ice protected from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
**Enhancer**: Ready to use as supplied. Keep on ice protected from light during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C.
**Lactate Dehydrogenase Standard (100 U/mL)**: Dilute the Lactate Dehydrogenase Standard to 20 U/mL by adding 200 µL of the Lactate Dehydrogenase Standard to 800 µL of Assay Buffer, mix well. Keep on ice and protect from light during the assay. Aliquot standard so that you have enough volume to perform the desired number of assays. Store aliquots at - 20°C.

### Standard preparation

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours

Using 20 U/mL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

| | Volume of 20 µM Standard | Assay Buffer | Concentration |
|---|---|---|---|
| Std.1 | 200 µL | 0 µL | 20 U/mL |
| Std.2 | 160 µL | 40 µL | 16 U/mL |
| Std.3 | 120 µL | 80 µL | 12 U/mL |
| Std.4 | 80 µL | 120 µL | 8 U/mL |
| Std.5 | 40 µL | 160 µL | 4 U/mL |
| Std.6 | 20 µL | 180 µL | 2 U/mL |
| Std.7 | 10 µL | 190 µL | 1 U/mL |
| Blank | 0 | 200 µL | 0 |

### Sample preparation

*Note: We recommend performing several dilutions of your sample to ensure the readings are within the standard value range. We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C for one month.*

Homogenize 0.1 g Tissues, or 5 x $10^6$ Cells, or 0.4 mL Erythrocytes on ice in 1 mL cold assay buffer; Centrifuge at 10,000 x g for 15 min at 4°C; Collect the supernatant for assay and store on ice. Serum can be tested directly. Add 2-50 µL samples into a 96-well plate; bring the volume to 50 µL with Assay Buffer.

### Assay procedure

1. The Working Reagent Preparation. For each well of reaction, prepare 55 µL Working Reagent by mixing 31 µL Assay Buffer, 8 µL NAD, 5 µL WST-8, 1 µL Enhancer and 10 µL Lactate. Fresh reconstitution is recommended.
2. Reaction. Add 50 µL of diluted standard and sample per well. Then add 50 µL Working Reagent to each sample and standard well (multi-channel pipettor is recommended). Tap plate to mix. Immediately read optical density at 450nm ($OD_0$).
3. Incubate for 30 min at 37°C in the dark. Read optical density at 450nm again ($OD_{30}$).

## DATA ANALYSIS

## Calculation of results

1. For each standard and sample well, calculate $\Delta OD = OD_{30} - OD_0$
2. Subtract blank ΔOD from the standard ΔOD values and plot the ΔOD against standard concentrations.
3. Determine the slope using linear regression fitting. The LDH concentration of Sample is calculated as

$$[LDH] = \frac{\Delta OD_{SAMPLE} - \Delta OD_{BLANK}}{Slope} \times n \text{ (U/mL)}$$

Note: If the $\Delta OD_{SAMPLE}$ values are higher than the ΔOD value for the 20U/mL standard, dilute sample in Assay Buffer and repeat this assay. Multiply the results by the dilution factor.

## Typical data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.