Glycolysis Assay Kit (Colorimetric)

LS-K231-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Introduction

GLYCOLYSIS is one of the major metabolic pathways cells undergo to produce energy and results in the production of pyruvate. One of the eventual fates of pyruvate from this process is lactate dehydrogenase converting it to L-lactate via lactic acid fermentation allowing L-lactate to serve as an indicator of glycolysis. LSBio's Glycolysis assay kit is based on measuring the production of L-Lactate from glycolysis in cells. L-Lactate that is secreted into the cell media is quantified using a coupled reaction involving the lactate dehydrogenase catalyzed oxidation of L-lactate that generates pyruvate and NADH which reduces a formazan dye. The intensity of the reduced dye, measured at 565 nm, is directly proportional to the L-lactate concentration in the sample, which in turn is directly proportional to the glycolytic rate of the cells.

Key Features

- Fast and sensitive. Use of 5 µL sample. Linear detection range up to 10 mM L-lactate in 96-well plate assay.
- Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 30 minutes. Room temperature assay. No 37°C heater is needed.
- High-throughput. "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Applications

- Direct Assays: L-Lactate produced by glycolysis in cell samples. Screening of glycolysis inhibitors and effect of drugs on glycolysis.

Components

Component	K231-100
	100 Tests
Assay Buffer	12 mL
Enzyme A	120 µL
Enzyme B	120 µL
NAD/MTT	1 mL
Standard (0.5M L-Lactate)	250 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, and plate reader.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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Assay Procedure

Sample Preparation: Plate cells in media of choice. After allowed enough time for cells to adhere to plate, remove media and replace with low percentage FBS media (FBS ≤ 1% or serum free media). For suspension cells, seed at desired cell number in low percentage FBS media. Set 2 mL of media aside for making standards. Add any treatments or compounds being tested at this step as well. Allow cells to propagate to desired confluence. Remove media for assay.

Procedure using 96-well plate

1. Standards. Prepare 500 µL of 10 mM Premix by mixing 10 µL of the Standard (0.5 M) and 490 µL of the low percentage FBS media used for the cells. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + Media	L-Lactate (mM)
1	100 µL + 0 µL	10
2	60 µL + 40 µL	6
3	30 µL + 70 µL	3
4	0 µL + 100 µL	0

2. Transfer 5 µL standards into separate wells of a clear, flat-bottom 96- well plate. Transfer 5 µL of each sample into separate wells. (For improved accuracy, we recommend running all wells in at least duplicate)
3. Prepare sufficient Working Reagent (WR) by mixing for each standard and sample well, 95 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme B and 8 µL NAD/MTT. Fresh reconstitution of the WR is recommended.
4. Add 95 µL Reagent to the four Standards and the Sample Wells. Tap plate to mix briefly and thoroughly. Incubate 30 minutes at room temperature.
5. Read optical density at 565 nm (520-600 nm).

Calculation:

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the L-Lactate concentration of Sample as follows:

$$[\text{L-Lactate}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (mM^{-1})} \quad (mM)$$

OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Media Blank (#4), respectively.

Conversions: 1 mM L-lactate equals 9.01 mg/dL, or 90.1 ppm.

Note: This assay reaches OD values greater than 1.0. If your plate reader is not accurate to values that high, you may choose to construct a modified 0, 1.5, 3, 5 mM standard curve instead.

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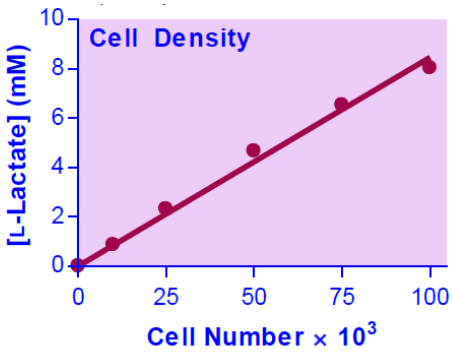
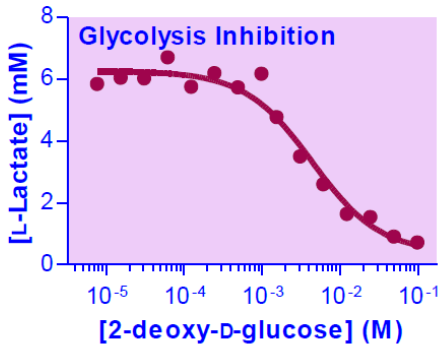
Sample Data



Cell Density and Rate of Glycolysis
HL-60 cells seeded at varying cell densities in RPMI medium



Glycolysis Inhibition
PANC1 cells treated with varying concentrations of 2-deoxy-D-glucose in DMEM medium

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