

EnzyChrom™ Glycolysis Assay Kit (ECGL-100)

Quantitative Colorimetric Determination of Glycolysis

DESCRIPTION

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.

BioAssay Systems' 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.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 12 mL **Enzyme A:** 120 µL
NAD/MTT: 1 mL **Enzyme B:** 120 µL
Standard: 250 µL 0.5M L-Lactate

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

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

- 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)
- 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.

- 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.
- 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:

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

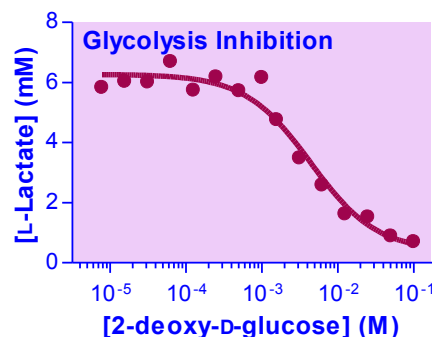
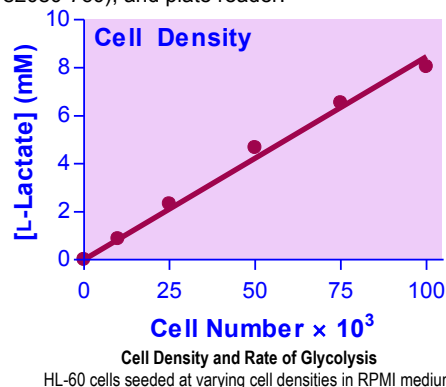
OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Media Blank (#4), respectively.

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



LITERATURE

- TeSlaa, T., et al (2014). Techniques to Monitor Glycolysis. *Methods Enzymol* 542: 91-114.
- Kim, J., et al (2006). Cancer's Molecular Sweet Tooth and the Warburg Effect. *Cancer Res* 66.18: 8927-8930.
- Rogatzki, M., et al (2015). Lactate is Always the End Product of Glycolysis. *Front Neurosci* 9: 22.