Glycolysis Assay Kit (Colorimetric)

Glycolysis Assay Kit (Colorimetric) is a detection kit for the quantification of Glycolysis in cell culture supernatants.

Catalog number: ARG82168

Package: 100 tests

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INTRODUCTION

Glycolysis is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvic acid, $CH_3COCOOH$. The free energy released in this process is used to form the high-energy molecules adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH). Glycolysis is a sequence of ten reactions catalyzed by enzymes.

Glycolysis is a metabolic pathway that does not require oxygen. The wide occurrence of glycolysis in other species indicates that it is an ancient metabolic pathway. Indeed, the reactions that make up glycolysis and its parallel pathway, the pentose phosphate pathway, occur in the oxygen-free conditions of the Archean oceans, also in the absence of enzymes, catalyzed by metal. [Provide by Wikipedia: Glycolysis]

PRINCIPLE OF THE ASSAY

This Glycolysis Assay Kit (Colorimetric) is a simple colorimetric assay that measures 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 O.D. 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.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Assay Buffer	12 mL	-20°C
Enzyme A	120 µL	-20°C
Enzyme B	120 µL	-20°C
NAD/MTT	1 mL	-20°C
Standard (0.5 M L-Lactate)	250 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 565 nm
- Centrifuge and centrifuge tube
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell culture supernatant: 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. Collect media for assay.

REAGENT PREPARATION

- **Working Reagent:** for each assay, mix 95 µL of Assay Buffer, 1 µL of Enzyme A, 1 µL of Enzyme B and 8 µL of NAD/MTT. Prepare immediately before assay.
- **Standards:** mix 10 µL of 0.5 M Standard with 490 µL of low percentage FBS media used for the cells. (Standard Premix final conc. is 10 mM). Dilute standards as follows.

Standard tube	L-Lactate (mM)	Low percentage FBS media (µL)	Standard Premix, 10 mM (µL)
S1	10	0	100
S2	6	40	60
S3	3	70	30
S4	0	100	0

ASSAY PROCEDURE

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening.

	Standard well	Sample well
Standard	5 µL	
Sample		5 µL
Working Reagent	95 µL	95 µL
Tap plate to mix briefly and thoroughly. Incubate for **30 minutes** at **room temperature**.		
Read the absorbance at **O.D. 565 nm**. (520-600 nm)		

Note:

- For improved accuracy, we recommend running all wells in at least duplicate.
- 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.

CALCULATION OF RESULTS

1. Subtract blank value (S4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the L-Lactate concentration of Sample as follows:

   L-Lactate (mM) = [(OD_{Sample} – OD_{Blank}) / Slope (mM^{-1})]

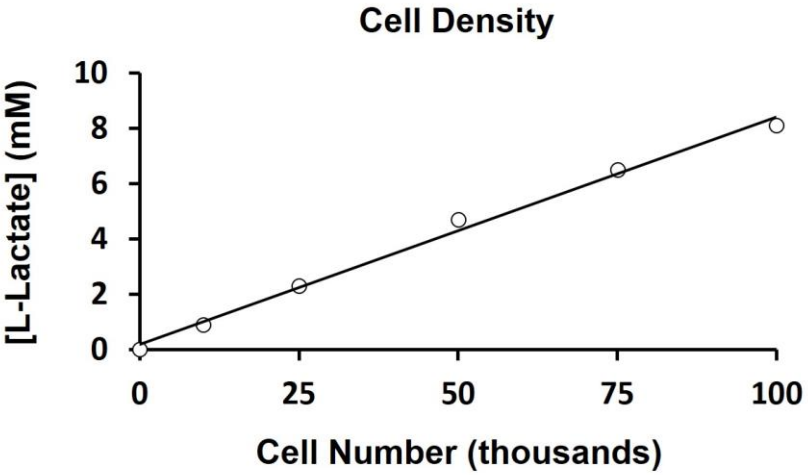
   Note:

   - OD_{Sample}, OD_{Blank}: the O.D. 565 nm values of the sample and media blank (S4), respectively.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Glycolysis Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



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

QUALITY ASSURANCE

Sensitivity

1.5 mM