Glycolysis

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1338

(Version 1.1A)

Detection and Quantification of Glycolysis Based on L-Lactate Produced in Cell Culture Supernatant.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Glycolysis is a fundamental metabolic pathway that converts glucose to pyruvate while producing ATP and NADH. Pyruvate generated through this route can be reduced to L-lactate by lactate dehydrogenase, a reaction that regenerates NAD^+ and permits continuous glycolytic operation. Because lactate output is stoichiometrically linked to glycolytic throughput, measuring extracellular L-lactate provides a direct, quantitative index of cellular glycolytic activity.

Glycolysis Colorimetric Microplate Assay Kit is a sensitive assay for measuring L-lactate produced from glycolysis in cells. The kit is based on L-lactate dehydrogenase catalyzed oxidation of L-lactate, in which the formed NADH reduces a formazan reagent. The intensity of the product color, measured at 450 nm, is proportionate to the L-lactate concentration in the sample, which in turn is directly proportional to the glycolytic rate of the cells.

II. KIT COMPONENTS

Component	100 Assays	Storage
Reaction Buffer	10 ml x 1	4 °C
Diluent	2 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Coenzyme	Powder x 1	-20 °C, keep in dark
Dye Reagent A	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Incubator (5 % CO_2, 90 % humidity, 37 °C)
2. Inverted optical microscope
3. PBS
4. Cell culture medium containing low percentage FBS (<1% or serum free)
5. Microplate reader to read absorbance at 450 nm
6. Distilled water
7. Pipettor, multi-channel pipettor
8. Pipette tips
9. 96-well culture plates
10. Centrifuge
11. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 100 mmol/L of standard stock solution, store at 4 °C for 1 month after reconstitution. Then dilute to 20 mmol/L standard top solution by adding 100 µl stock solution into 400 µl low FBS media. Perform 2-fold serial dilutions of the top standard solution using low FBS media to make the standard curve. The concentration of standard curve could be 20/10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Store at 4°C for 1-2 days or -20 °C for 1 month.

Coenzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Keep in dark and store at 4°C for 1 week or -20 °C for 2 months.

Dye Reagent A: Add 6 ml distilled water to dissolve before use, mix. Keep in dark and store at 4°C for 1 week or -20°C for 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

For Suspension Cells

1. Harvest cells and wash with pre-warmed PBS 1-2 times.

2. Adjust cell density to 2×10^5 - 8×10^5 cells/mL using low percentage FBS medium (<1% or serum free).

3. Incubate at 37 °C with 5% CO_2 for 1-24 h. Metabolic interventions or drug treatments can be applied at this step if needed.

4. After incubation, centrifuge the cell with media at 300×g for 5 min. Carefully transfer supernatant for assay.

For Adherent Cells

1. Seed cells when cells reach 70%-80% confluence (approximately 3×10^4 - 5×10^4 cells/cm^2).

2. Remove existing complete medium and wash cells gently 1-2 times with pre-warmed PBS.

3. Add low percentage FBS medium (<1% or serum free) and incubate at 37 °C with 5% CO_2 for 1-24 h. Metabolic interventions or drug treatments can be applied at this step if needed.

4. After incubation, centrifuge at 200×g for 5 min and aspirate the supernatant for assay.

Note:

1. Incubation Time depends on the glycolytic activity of the cells and density, medium volume, glycolytic rate, and assay sensitivity. If the optimal time point is unknown, perform a pilot study with multiple time points (e.g., 0, 0.5, 1, 2, 4, and 8 h) to confirm the linear range.

2. Collect supernatants for assay within 1-4 h after changing to cell starvation. Lactate accumulation is typically linear during this period.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	100 µl	100 µl	100 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Low FBS media	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Coenzyme	10 µl	10 µl	10 µl
Dye Reagent A	60 µl	60 µl	60 µl
Dye Reagent B	10 µl	10 µl	10 µl
Mix, keep at room temperature for 30 minutes, record absorbance measured at 450nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VII. CALCULATION

1. Plot the standard curve with the concentration of standard on the x-axis and the $OD_{(Sample)} - OD_{(Blank)}$ on the y-axis. The trendline format can be set to Power as follow.

$y = ax^b$

2. Calculate the sample concentration in ASSAY PROCEDURE according to the Coefficient(a) and Power(b) of the standard curve

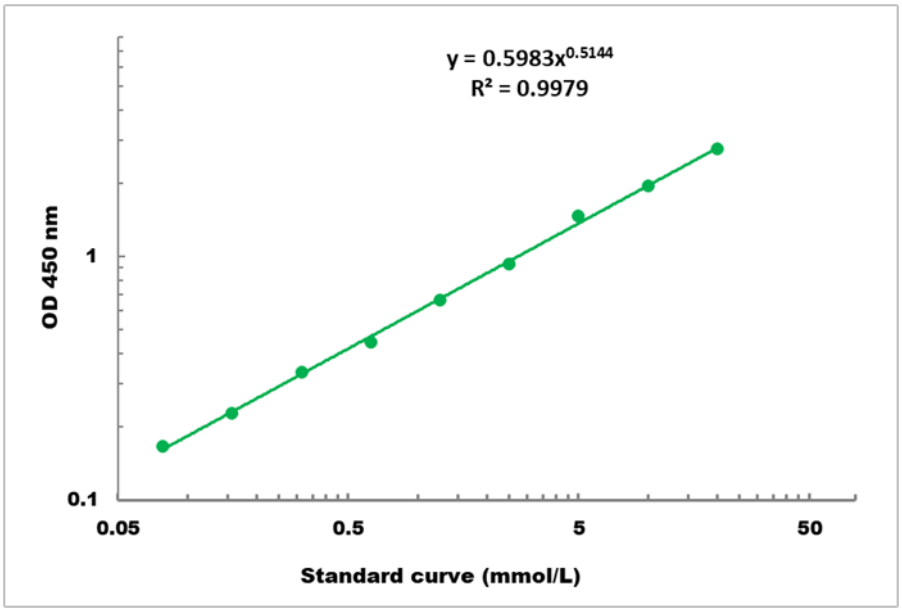
$$C_{Sample} = \sqrt[Power(b)]{\frac{OD_{Sample} - OD_{Blank}}{Coeffient(a)}} \quad (mmol/L)$$

a: The Coefficient of the standard curve

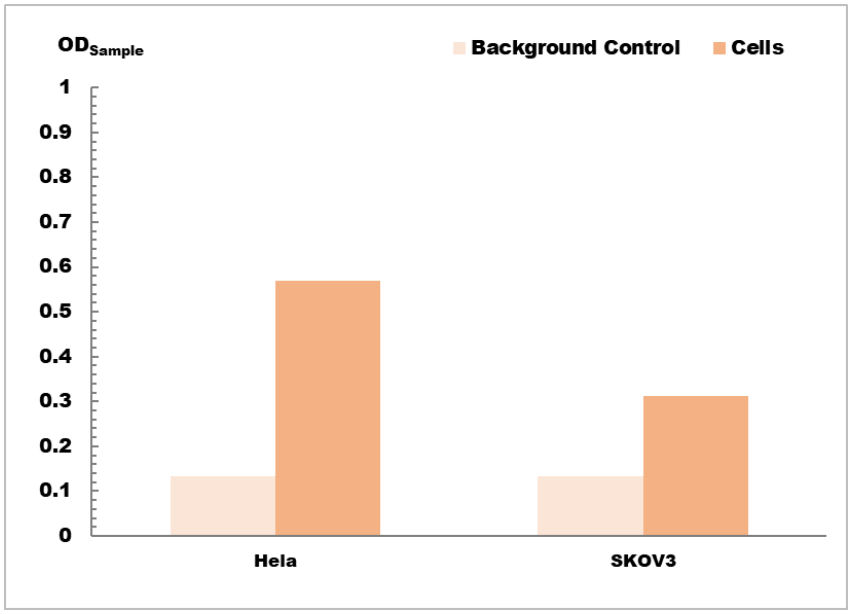
b: The Power of the standard curve

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 20 mmol/L



Determination of glycolytic rate in cell lines with different Glycolytic Capacity (both seeded at 3×10^4 cells/cm² and cultured to ~80% confluence)