Technical Bulletin

Glycolysis Assay Kit

Catalog Number MAK439

Product 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 end products of this process is lactate dehydrogenase-mediated conversion of pyruvate to L-lactate via lactic acid fermentation. This enzymatic mechanism allows L-lactate to serve as an indicator of glycolysis.

The 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 subsequently 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. The assay method has a linear response up to a concentration of 10 mM L-Lactate.

The Glycolysis Assay Kit is suitable for the direct determination of L-Lactate produced by glycolysis in cell media samples and for the screening of glycolysis inhibitors.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Assay Buffer 12 mL
  Catalog Number MAK439A
- Enzyme A 120 µL
  Catalog Number MAK439B
- NAD/MTT 1 mL
  Catalog Number MAK439C
- Enzyme B 120 µL
  Catalog Number MAK439D
- Standard (0.5 M L-Lactate) 250 µL
  Catalog Number MAK439E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Cells of interest and appropriate culture media
- Fetal bovine serum (FBS), if required

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all assay reagents to room temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Adherent Cell Lines

1. Plate adherent cells in media of choice.
2. After allowing enough time for cells to adhere to plate, remove media and replace with low percentage FBS media (FBS ≤ 1%) or serum free media).

Suspension Cell Lines

1. Seed at desired cell number in low percentage FBS media (FBS ≤ 1%).

All Cell Lines

1. Reserve 2 mL of media for use in standard curve preparation.
2. Add any treatments or compounds being tested for effect on glycolysis to the cell culture.
3. Allow cells to propagate to desired confluence.
4. Remove media from the culture and retain for use in assay.
5. Transfer 5 µL of each media Sample to separate wells of a clear flat-bottom 96-well plate.

Standard Curve Preparation

Note: The Standard readings for this procedure includes A_{565} values greater than 1.0. If the plate reader being used is not accurate to values greater than 1.0, it is recommended to prepare a modified [0, 1.5, 3 and 5 mM] standard curve.

1. Prepare a 10 mM L-Lactate Standard by mixing 10 µL of the 0.5 M L-Lactate Standard with 490 µL of the same low percentage FBS media used for the cell culture.
2. Prepare L-Lactate standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of L-Lactate standards

Well	10 mM L-Lactate	Low FBS Media	L-Lactate (mM)
1	100 µL	-	10
2	60 µL	40 µL	6
3	30 µL	70 µL	3
4	-	100 µL	0

3. Mix well and transfer 5 µL of each Standard into separate wells of the plate.

Working Reagent

Mix enough reagent for the number of assays to be performed. For each Standard and Sample well, prepare 105 µL of Working Reagent according to Table 2.

Table 2.
Preparation of Working Reagent

Reagent	Working Reagent
Assay Buffer	95 µL
Enzyme A	1 µL
Enzyme B	1 µL
NAD/MTT	8 µL

Measurement

1. Add 95 µL of the Working Reagent to each Sample and Standard well. Tap plate to mix.
2. Incubate the plate for 30 minutes at room temperature.
3. Read the absorbance of all Samples and Standards at 565 nm (A_{565}).
4. If the sample reading is higher than the 10 mM Standard reading, dilute Sample in purified water and repeat the assay.

Results

1. Subtract the 0 Standard A_{565} reading from all standard A_{565} readings.
2. Plot the corrected A_{565} Standard readings against the Standard concentrations and determine the slope of the Standard curve.
3. Calculate the Sample L-Lactate concentration:

$$\text{L-Lactate (mM)} = \frac{(A_S - A_B) \times DF}{\text{Slope } (mM^{-1})}$$

where

A_S = A_{565} reading of Sample

A_B = A_{565} reading of Media Blank (Standard #4)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

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

Figure 1.
Cell Density and Rate of Glycolysis
HL-60 cells were seeded at varying cell densities in RPMI medium to demostrate the linear response of lactate generation.

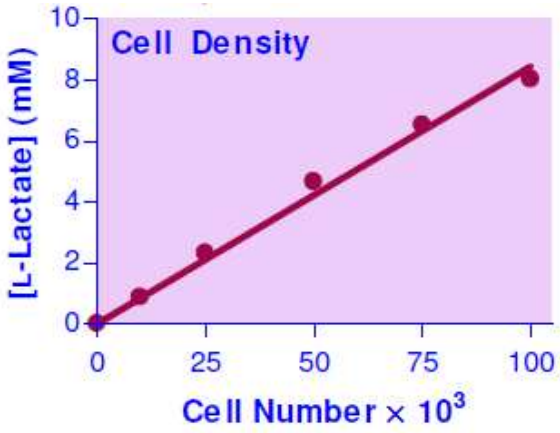
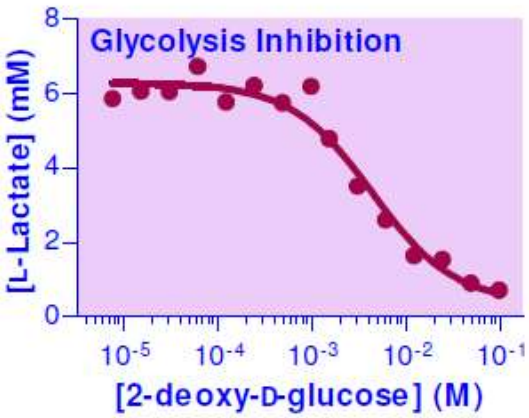


Figure 2.
Glycolysis Inhibition
PANC1 cells in DMEM medium were treated with varying concentrations of 2-deoxy-D-glucose to inhibit glycolysis.



References

1. Helsel, A.R., et al., Glycolysis-optimized conditions enhance maintenance of regenerative integrity in mouse spermatogonial stem cells during long-term culture. *Stem Cell Reports*, **8(5)**, 1430-1441 (2017).
2. Umezu, R., et al., Macrophage (Drp1) dynamin-related protein 1 accelerates intimal thickening after vascular injury. *Arterioscler. Thromb. Vasc. Biol*., **40(7)**, e214-e226 (2020).
3. Moschovaki Filippidou, F., et al., Glucagon-like peptide-1 receptor agonism improves nephrotoxic serum nephritis by inhibiting t-cell proliferation. *Am. J. Pathol*., **190(2)**, 400-411 (2020).

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