# Glycolysis Cell-Based Assay Kit

Item No. 600450

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# GENERAL INFORMATION

## Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

| Item Number | Item | Quantity/Size | Storage |
|---|---|---|---|
| 600451 | Glycolysis Assay Substrate | 1 vial/250 µl | -20°C |
| 600452 | Glycolysis Assay Enzyme Mixture | 2 vials | -20°C |
| 600454 | Glycolysis Assay Cofactor | 1 vial/250 µl | -20°C |
| 600455 | Glycolysis Assay L-Lactate Standard | 2 vials | -20°C |
| 10009322 | Cell-Based Assay Buffer Tablet | 1 tablet | RT |

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

## If You Have Problems

**Technical Service Contact Information**

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Email:** techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader with the capability to measure absorbance between 490-520 nm
2. A 96-well tissue culture-treated plate for culturing cells
3. A 96-well clear assay plate
4. A plate centrifuge
5. An orbital microplate shaker
6. Adjustable pipettes and a multichannel pipette
7. A source of pure water; glass-distilled water or HPLC-grade water is acceptable



# INTRODUCTION

## About This Assay

Cayman's Glycolysis Cell-Based Assay Kit provides a colorimetric method for detecting L-lactate, the end product of glycolysis, produced and secreted by cultured cells. In this assay, lactate dehydrogenase (LDH) catalyzes the reaction between $NAD^+$ and lactate, yielding pyruvate and NADH. The NADH directly reduces a tetrazolium salt (INT) to a colored formazan which absorbs between 490 and 520 nm. The quantity of formazan produced is proportional to the quantity of lactate in the culture medium and is thus an indirect measurement of glycolysis. This assay can be adapted to high-throughput screening to examine glycolytic regulators in a cell-based system. In addition, the non-invasive sampling leaves the cells intact and thus allows multiplex testing for additional markers.

# PRE-ASSAY PREPARATION

*NOTE: Fetal bovine serum (FBS) contains LDH and will generate a high background reading when used in the culture medium. We recommend that you use culture medium containing a low percentage of FBS (such as 0.25-1%) or serum-free culture medium for your experiments. The same culture medium without cells should be included in the assay as background controls.*

## Cell Culture Preparation

1. Seed cells in a 96-well tissue culture plate at a density of 1,000-50,000 cells/well in 120 µl of culture medium and grow the cells overnight in a $CO_2$ incubator at 37°C.
2. Set aside 2 ml of the same batch of culture medium used for cell growth. This medium will be used to prepare the L-lactate standards and will serve as a background control. It is best to incubate this medium at the same temperature and $CO_2$ concentration as the experimental cultures.
3. The next day, treat the cells with or without compounds to be tested. We recommend that each treatment be performed at least in duplicate.
4. Culture the cells in a $CO_2$ incubator at 37°C for 24 hours or for a period of time according to your typical experimental protocol.



## Reagent Preparation

**Some of the kit components are in lyophilized or concentrated form and need to be reconstituted or diluted prior to use. Follow the directions carefully to ensure proper volumes of assay buffer are used to reconstitute or dilute the vial components.**

1. **Assay Buffer Preparation**

   Dissolve the Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of pure water. This buffer should be stable for approximately one year at room temperature.

2. **Glycolysis Assay Enzyme Mixture - (Item No. 600452)**

   Each vial contains a lyophilized powder of enzymes. Immediately prior to use, reconstitute the contents of one vial with 150 µl of Assay Buffer. Keep the reconstituted Glycolysis Assay Enzyme Mixture on ice and use within one hour.

3. **Reaction Solution**

   To make 12 ml of reaction solution sufficient for use on one 96-well plate, add 120 µl of each of the following mixtures to 11.64 ml of the Assay Buffer:

   Glycolysis Assay Substrate (Item No. 600451)

   Glycolysis Assay Cofactor (Item No. 600454)

   Reconstituted Glycolysis Assay Enzyme Mixture (prepared in Step 2)

   The reaction solution is stable for approximately one hour at room temperature.

# ASSAY PROTOCOL

## Standard Preparation

### Glycolysis Assay L-Lactate Standard

To prepare the standard for use in the glycolysis assay, obtain seven clean test tubes or microcentrifuge tubes and label them #2 through #8. Aliquot 100 µl of culture medium that was previously set aside (see step 2 of the **Pre-Assay Preparation** section on page 6) into tubes #2-#8. Add 500 µl of the previously set aside culture medium to the 3 ml glass vial containing the Glycolysis Assay L-Lactate Standard (Item No. 600455). Vortex thoroughly to ensure the L-lactate is in solution. This 10 mM L-lactate solution is the first point on the standard curve. Serially dilute the standard by transferring 100 µl of the 10 mM stock into tube #2, mix thoroughly. Next, transfer 100 µl from tube #2 into tube #3, mix thoroughly. Repeat this procedure for tubes #4-#7. Do not add any standard to tube #8; this sample of culture medium will be your blank.

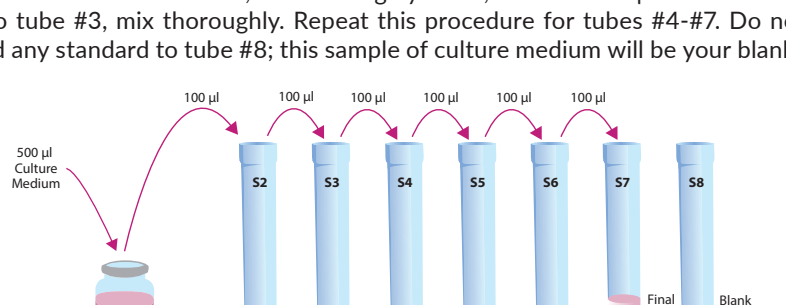



**Figure 1. Preparation of the L-lactate standards**

## Plate Set Up

There is no specific pattern for using the wells on the plate. However, a lactate standard curve in duplicate has to be assayed with the samples. We suggest that each sample be assayed at least in duplicate. A typical layout of standards and samples is given below (see Figure 2). We suggest that you record the contents of each well on the template sheet provided (see page 18).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | S1 | S1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | S2 | S2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | S3 | S3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | S4 | S4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | S5 | S5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | S6 | S6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | S7 | S7 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | S8 | S8 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |

S1-S8 = Standards
1-40 = Sample Wells

**Figure 2. Sample plate format**

## Performing the Assay

**Pipetting Hints**

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

1. Centrifuge the 96-well cell culture plate at 1,000 rpm for five minutes.
2. Using a new 96-well clear assay plate, add 90 µl of Assay Buffer to each well.
3. Transfer 10 µl of the standards prepared above into the appropriate wells. We recommend that the standards be run in duplicate.
4. Transfer 10 µl of supernatant from each well of the cultured cell plate to corresponding wells on the new plate. *NOTE: The supernatant may be diluted if a high rate of glycolysis is anticipated. Use the dilution factor in the L-lactate level calculation.*
5. Add 100 µl of reaction solution (prepared in step 3, on page 7) to each well, including the standard wells, using a multichannel pipette.
6. Incubate the plate with gentle shaking on an orbital shaker for 30 minutes at room temperature.
7. Read the absorbance at 490 nm with a plate reader.



# ANALYSIS

## Calculations

1. Determine the average absorbance of each standard and sample.
2. Subtract the absorbance value of the blank from itself and all other standards and samples. This is the corrected absorbance.
3. Plot the corrected absorbance values of each standard as a function of the final concentration of lactate. See Figure 3, on page 13, for a typical standard curve.
4. Calculate the L-lactate concentration of the samples using the corrected absorbance of each sample and the equation below.

$$\text{L-Lactate (mM)} = \left[\frac{\text{Absorbance - (y-intercept)}}{\text{Slope}}\right] \text{x Dilution Factor}$$

## Performance Characteristics

**Specificity:**

To assess substrate specificity, the assay was performed with L-lactate replaced by the structurally similar compound D-lactate. No reaction occurred when D-lactate was used at concentrations up to 10 mM.

The standard curve presented here is an example of the data typically produced with this assay; however, your results will not be identical to these. You must run a new standard curve with each experiment.

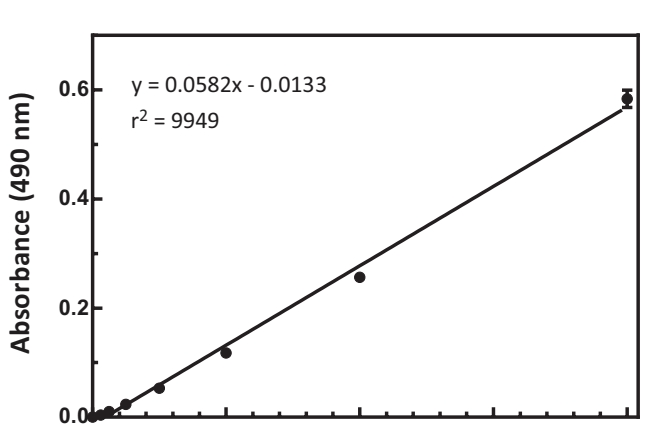



**Figure 3. L-Lactate standard curve**

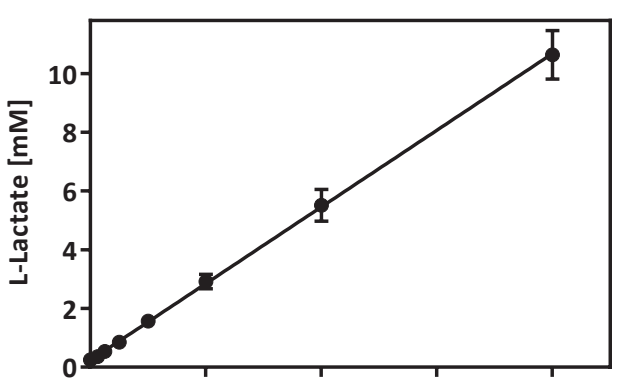



**Figure 4. Glycolysis levels can be accurately measured over a broad range of cell densities.** A two-fold dilution series of HeLa cells was seeded in triplicate to a 96-well plate with a starting density of 4 x $10^4$ cells per well. Cells were grown for four hours in DMEM containing 10% FBS at 37°C, 5% $CO_2$. Media was removed and replaced with 100 µl of DMEM containing 1% FBS. Cells were grown for 18 hours and assayed to determine the L-lactate concentration.

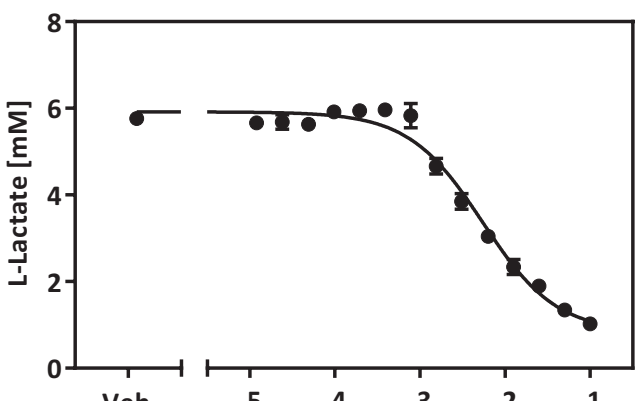



**Figure 5. 2-deoxy-D-glucose inhibition of HeLa cell glycolysis.** HeLa cells were seeded in a 96-well plate at a density of 25,000 cells/well in DMEM containing 10% FBS and grown in an incubator containing 5% $CO_2$ at 37°C. After four hours of incubation, the media was exchanged with DMEM containing 1% FBS and various concentrations of 2-deoxy-D-glucose. After 18 hours of incubation, the supernatants were assayed to determine the L-lactate concentrations.

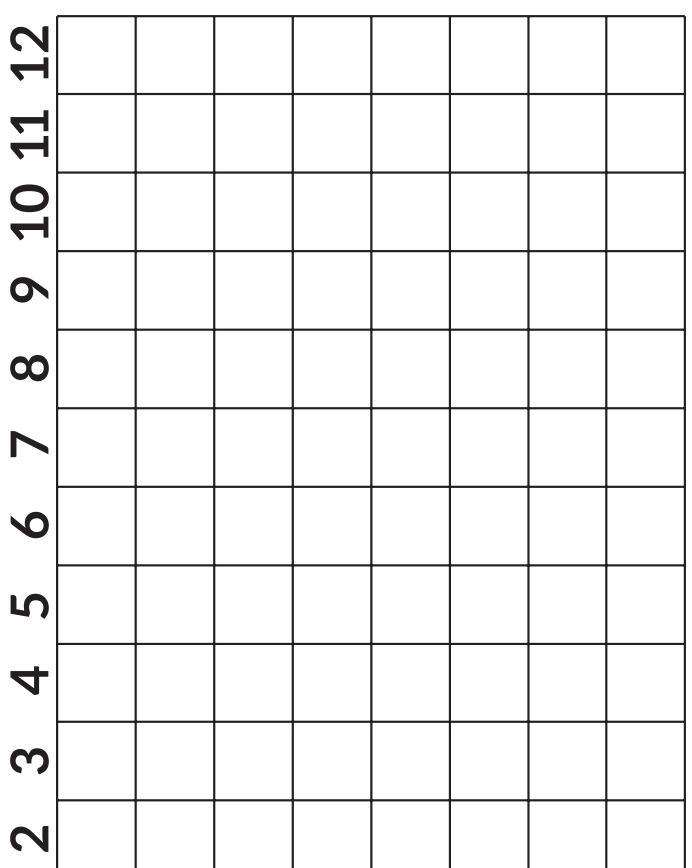
# RESOURCES

## Troubleshooting

| Problem | Possible Causes | Recommended Solutions |
|---|---|---|
| Erratic values; dispersion of duplicates | A. Poor pipetting/technique<br>B. Bubble in the well(s) | A. Be careful not to splash the contents of the wells<br>B. Carefully tap the side of the plate with your finger to remove bubbles |
| No signal detected above background in the sample wells | Sample was too dilute | Re-assay the sample using a lower dilution |
| The absorbance values of the sample wells were higher than the last standard | Sample was too concentrated | Re-assay the sample using a higher dilution |

## References


1. Demetrius, L.A., Coy, J.F., and Tuszynski, J.A. Cancer proliferation and therapy: The Warburg effect and quantum metabolism. *Theoretical Biology and Medical Modeling* **7**, 2-14 (2010).
2. Griffin, J.L. and Shockcor, J.P. Metabolic profiles of cancer cells. *Cancer* **4**, 551-561 (2004).
3. McCarty, M.F. and Whitaker, J. Manipulating tumor acidification as a cancer treatment strategy. *Altern. Med. Rev.* **15(3)**, 264-272 (2010).
4. Kondoh, H., Lleonart, M.E., Nakashima, Y., *et al.* A common metabolic profile shared between murine ES cells and primary cells bypassing senescence. *Medical Hypothesis and Research* **4(1)**, 29-36 (2008).
5. Barger, J.F. and Plas, D.R. Balancing biosynthesis and bioenergetics: Metabolic programs in oncogenesis. *Endocrine-Related Cancer* **17**, R287-R304 (2010).






## NOTES

## Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.