Bioluminescent Assays Facilitate Screening for Effectors of Glucose Metabolism

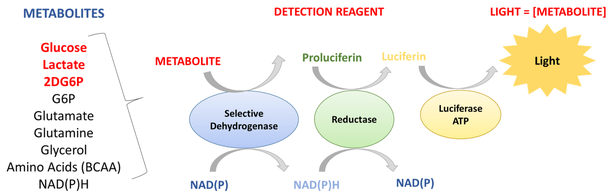
Richard Somberg, Donna Leippe, Michael P. Valley, Natasha Karassina, Mary Sobol, Gediminas Vidugiris and Jolanta Vidugiriene

Promega Corporation, 2800 Woods Hollow Rd, Madison, WI, 53711

Abstract #290755

1. Introduction

Cellular metabolism is of increasing interest in many areas of research including the fields of cancer, diabetes, immunology, aging and neuroscience. This has increased the need for new technologies that can be used to measure key metabolites, especially plate-based assays that are amenable to higher throughput formats and can facilitate testing several samples or the screening of several compounds. In this poster, we describe bioluminescent assays for measuring metabolites and focus on three that can be used to study glucose metabolism and compounds that affect it: glucose, lactate and glucose uptake assays.



- Metabolite Assays use selective dehydrogenases to produce NAD(P)H in proportion to the amount of metabolite
- NAD(P)H is detected using Reductase enzyme and its Proluciferin substrate in combination with luciferase enzyme to produce light

2. Glucose and Lactate Detection Assays

These are sensitive and rapid assays that work with various sample types (cells, tissues, plasma) with minimal sample preparation, and are amenable to lower volume, higher-throughput protocols

Example Protocol For Assay in Low Volume 384-well plates

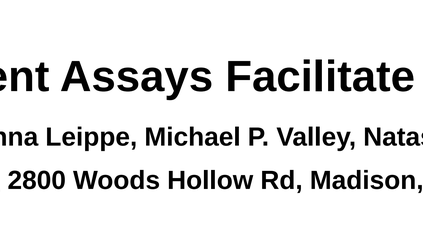
9µl Sample containing metabolite(s)

+

9µl Metabolite Detection Reagent

Incubate 1 hour at room temperature

Record Luminescence



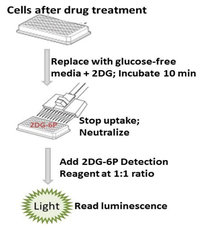
	Glucose	Lactate
Sensitivity: L.O.D. at S/N= 33	100nM	400nM
Linearity	to 50µM	to 100µM
Assay Window = S/Bmax	>500	>150

3. Glucose Uptake Assay

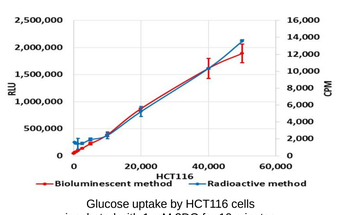
Glucose uptake is traditionally measured using a radioactive glucose analog, 2-deoxyglucose (2DG). Once in the cell, 2DG is phosphorylated but not further metabolized, resulting in the the accumulation of a stable and impermeable metabolite 2-deoxyglucose-6-phosphate (2DG6P). With the non-radioactive bioluminescent assay, 2DG6P is detected using a selective dehydrogenase, glucose-6-phosphate dehydrogenase (G6PDH), and the NAD(P)H detection technology described above.

- Sensitive: L.O.D. ~200nM 2DG6P
- Broad linearity: Linear to 40µM 2DG6P
- Large Signal Window: S/Bmax > 100-fold

Simple protocol



Comparable performance of radioactive and bioluminescent methods

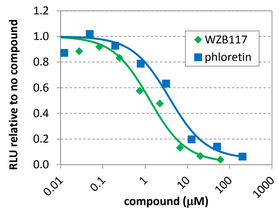


Glucose uptake by HCT116 cells incubated with 1 mM 2DG for 10 minutes

4. Glucose Transporter Inhibition

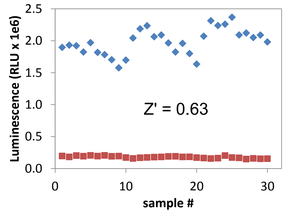
The higher proliferation rates of cancer cells are fueled by an increase in glycolysis. This is achieved by the overexpression of glucose transporters to increase glucose uptake, making glucose uptake an attractive pharmacological target. The glucose uptake assay can be used to monitor the affects of compounds that interfere with this process.

Inhibition of Glucose Uptake



HCT116 cells were plated at 10,000 cells per well and treated for 10 minutes with a titration of glucose transporter inhibitors (in 50µl). Five microliters of 10 mM 2DG was added for 10 minutes for glucose uptake measurements.

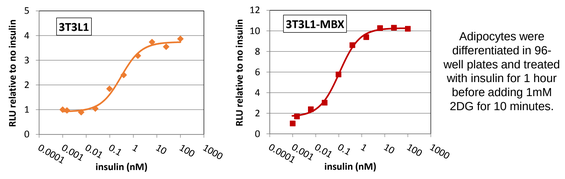
Z-factor in 96-well plate



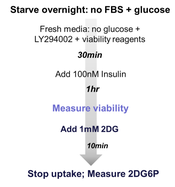
HCT116 cells were plated at 20,000 cells per well in 60 wells of a 96-well plate. After treating with 25µl PBS +/- 50µM cytochalasin B (30 wells each) for 5 minutes, 25µl PBS + 2mM 2DG was added to begin the glucose uptake measurements. Red squares: with cytochalasin; Blue diamonds: no cytochalasin

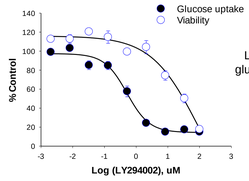
5. Insulin Stimulation of Glucose Uptake in Adipocytes

3T3-L1 cells are commonly differentiated into adipocytes as a model to study the effects of insulin on fat cells. While a four-fold stimulation of glucose uptake by insulin is typically seen with these cells, we have also used the MBX cell variant which exhibits closer to ten-fold stimulation with a similar insulin EC_{50}.



Adipocytes were differentiated in 96-well plates and treated with insulin for 1 hour before adding 1mM 2DG for 10 minutes.

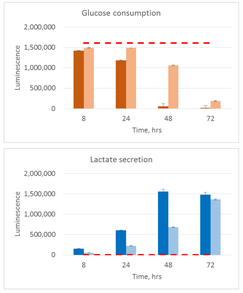


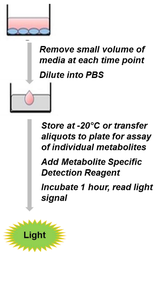


PI3K inhibitor LY294002 inhibited glucose uptake by 3T3 L1-MBX cells

6. Glucose Consumption and Lactate Secretion by Cancer Cells

Cancer cells consume glucose and secrete lactate which can be monitored over time by removing a small aliquot of cell culture medium.
Samples from cells at different cell densities and different time points can be frozen and analyzed using one dilution factor, providing flexible and convenient experimental setup and easier data analysis.



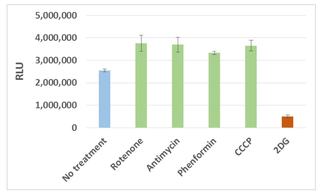


A549 cells were plated at 15K (dark bars) or 5K (light bars) cells per well in 100µL DMEM with 5mM glucose, 2mM glutamine and 10% dialyzed serum. At the indicated time points, 2.5µl of medium was removed, diluted in 97.5µl PBS and frozen until all samples had been collected.

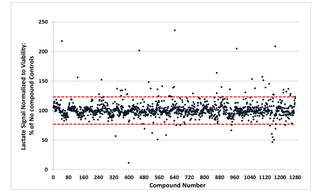
7. Homogeneous Assay for Effectors of Glucose Metabolism

Lactate production by cells and changes caused by treatments can quickly be monitored using an in-well homogeneous assay protocol. Cells were incubated with compounds for 1 to 2 hours and lysed before addition of lactate detection reagent.

Effect of compounds on glycolysis as measured by lactate production



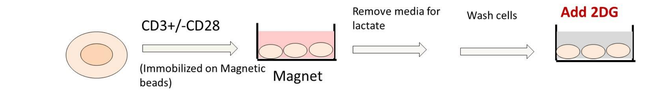
A549 cells were plated at 15,000 cells/well in defined medium in 96 well plates. The cells were incubated with compounds for 1hr at 37°C before lactate production was measured. Compounds tested included the glycolysis inhibitor 2DG (10mM) and four mitochondrial inhibitors (5µM rotenone, 5µM antimycin, 2.5mM phenformin and 50µM CCCP).

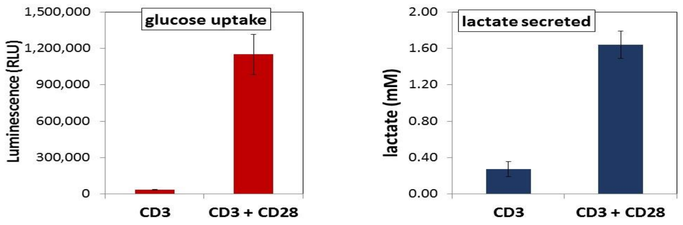


Compounds of the LOPAC^{1280} library (Sigma) were screened in low volume 384-well plates. SKOV-3 cells (1000 cells/well) were incubated with compounds for 1hr at 37°C before lactate production was measured. Signals were normalized to viability readings made from the same well using a bioluminescent real-time viability reagent before calculating percentage of lactate production as compared to wells that received no compound. Z' values for all plates in this experiment were > 0.6.

8. Immunometabolism: Activation of T Cells

Increase in glycolysis upon activation of T cells results in an increase in glucose uptake and an increase in lactate secretion





Glucose uptake and lactate secretion by human peripheral blood T cells was measured after incubating cells (5000 in 100µl medium per well) with CD3 +/-CD28 beads for 24 hours at 37°C.

9. Conclusions

- **Cellular energy metabolism is important for proper cell function**
  - It is a therapeutic target for many diseases and of interest for many areas of research
- **Bioluminescent metabolite assays can facilitate the study of cellular energy metabolism**
  - Assays for measuring glucose, glucose uptake and lactate can be useful for studies of glycolysis
  - Nonradioactive glucose uptake measurements
  - Plate assay suitable for higher-throughput plate formats and protocols
  - Multiplexing with cell viability assays provide more information per well and facilitates data normalization
- **Glucose and Lactate Assay Features**
  - Flexibility for different sample types with minimal sample preparation
  - Sensitivity enables studying metabolites using small numbers of cells per well (e.g. 1000 cells/well)
  - Broad linearity of 2 to 3 logs provides convenience for testing samples at different metabolite concentrations
  - Wide assay windows with S/Bmax > 100 allows for better discrimination of small changes

Corresponding author: donna.leippe@promega.com