# Sensitive Bioluminescent Assays for Monitoring Changes in Tumor and Immune Cell Metabolism

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**Abstract # 5272**

## 1. Introduction

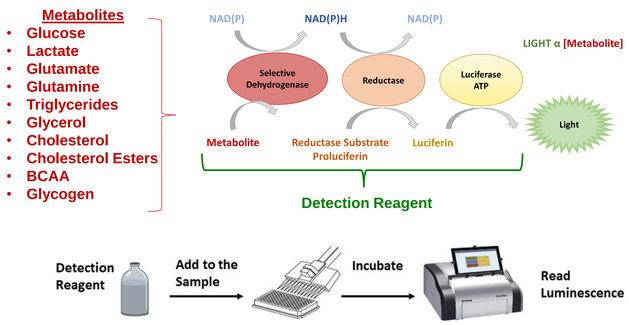
Cells of the tumor microenvironment must compete for fuels and respond to changing, dysregulated conditions to remain viable and functional. Identifying the metabolic requirements and vulnerabilities of tumor and immune cells in this environment can aid in the development of more effective cancer treatments.

We have developed a series of bioluminescent metabolite detection assays for monitoring key cell metabolic pathways such as: glycolysis by glucose and lactate detection; glutaminolysis with glutamine and glutamate measurements; and lipolysis and lipogenesis through glycerol and triglyceride measurements. The assays share a common NAD(P)H bioluminescent detection technology and were developed to have high sensitivity, an important feature when samples are limiting and contain only a few cells or small volumes.

We have used these assays to study the metabolic activity of tumor cell lines and T cells. Here we present an example of studying T cell activation *in vitro,* and its fuel requirements, by monitoring a switch to glycolysis and an increase in lactate secretion over time. We also used the assays to follow metabolic pathway alterations induced in cancer cell lines by treatment with small molecules. Treatment with such compounds might provide a means of manipulating tumor nutrient usage and metabolite secretion in the tumor microenvironment.

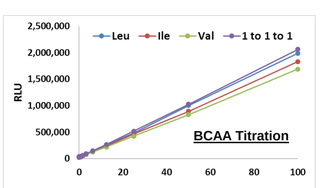
## 2. Bioluminescent Metabolite Assays

- The bioluminescent metabolite assays use a core bioluminescent NAD(P)H detection technology
- The technology uses coupled- enzyme reactions and a novel proluciferin substrate to generate a light signal proportional to the starting metabolite concentration

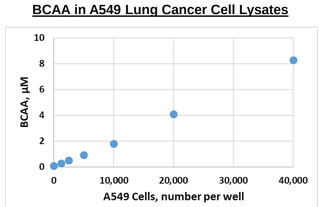


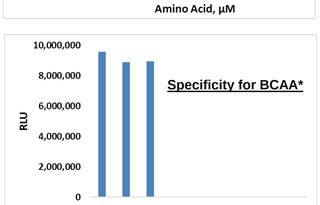

## 3. Branched-Chain Amino Acids

- The branched-chain amino acid (BCAA) assay can be used to measure leucine, isoleucine and valine amino acids
- With the improved assay sensitivity, BCAA can be measured in cell lysates prepared directly from cells plated in 96-well plates




**BCAA in A549 Lung Cancer Cell Lysates**

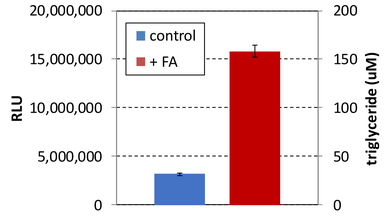



*Determined using 1 mM of each aa
The signals for non-BCAA aa's were at background (~40,000 RLU)

## 4. Triglyceride Accumulation in Liver Cancer Cells

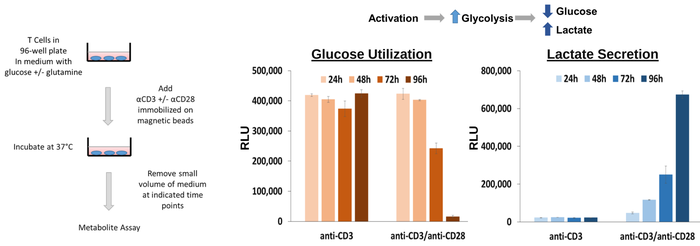
- Triglycerides can be measured in cells by lysing cells and digesting triglycerides to release glycerol
- Cells can be lysed directly in the well of 96-well plates using a non-organic lysis solution containing lipase




HepG2 cells (20,000 cells/well) were plated in a 96-well plate in complete medium. The medium was removed and replaced with serum-free media in the absence (blue bar, control) or presence (red bar, +FA) of 0.3 mM BSA-bound linoleic and oleic acids. After an overnight incubation, the medium was removed and the cells were washed and lysed using lipase-containing lysis solution. Lipid accumulation was then measured using the triglyceride detection assay.

## 5. T Cell Activation and Induction of Glycolysis

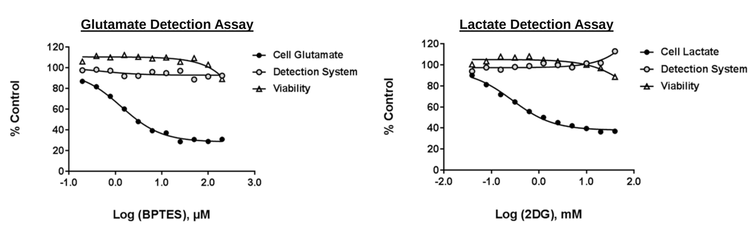
- Glycolysis is induced upon T cell activation and can be followed through an increase in lactate secretion
- Both glucose and lactate levels in cell culture media were followed over time by removing small aliquots at the indicated time points
- Activation required binding to both CD3 and CD28 and the presence of glutamine (data not shown)




Human Peripheral Blood T cells (250,000 cells in 100µl media containing 5 mM glucose and +/- 2 mM glutamine) were activated using antibodies to CD3 and CD28 immobilized on magnetic beads. Samples of medium were removed at the indicated time points and used to measure glucose and lactate.

## 6. Rapid Analysis of Glucose and Glutamine Pathway Inhibitors

- Metabolic pathways respond to changes in the environment and can be altered by the presence of small compounds, such as enzyme inhibitors
- In these examples, treatment of cells with a glycolysis pathway inhibitor (2DG) or a glutaminase inhibitor (BPTES) rapidly decreased lactate or glutamate production, respectively



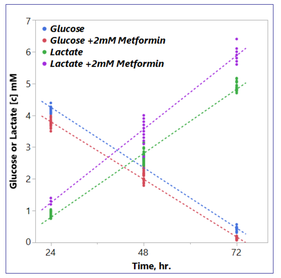

SKOV-3 ovarian cancer cells were incubated in 384-well plates with compounds for 1 hour at 37°C. Glutamate or lactate detection reagents were added directly to the wells of cells. The decrease in lactate or glutamate was not due to a loss of cell viability (RealTime-Glo™ MT Cell Viability Assay) or interference with the detection reagents (assayed with glutamate of lactate standards).

See Leippe D., *et al.* (2017) *SLAS Discovery* **22**:366-377

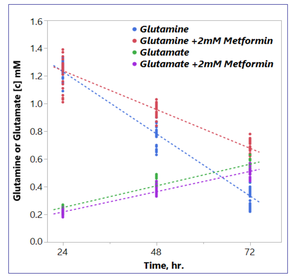
## 7. Metabolic Response to Inhibitors Over Time

- The effects of longer-term treatments can be tracked by determining changes in metabolites in media over time
- Metformin is a mitochondrial inhibitor that targets Complex I of the ETC
- In response to treatment with metformin and the shut-down of oxidative phosphorylation, A549 lung cancer cells increased aerobic glycolysis and altered glutamine metabolism

**Glucose and Lactate**




**Glutamine and Glutamate**

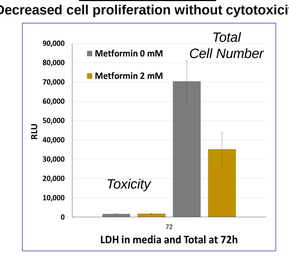



| Fold increase with 2 mM metformin at 72 hrs (after normalization for cell number) | |
|---|---|
| Glucose Consumption | 2.0 |
| Lactate Secretion | 2.4 |
| Glutamine Consumption | 1.3 |
| Glutamate Secretion | 1.8 |

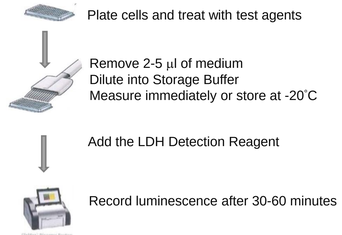
## 8. Multiplexing with Assays for Cytotoxicity and Cell Number

- The leakage of cytoplasmic lactate dehydrogenase (LDH) is a widely accepted and commonly used method to estimate the number of non-viable cells
- The NAD(P)H detection technology was extended to create a bioluminescent LDH activity assay
- The assay was used to investigate the cell health effects of metformin by quantitating LDH release into the medium (toxicity) as well as total LDH in cells lysed at the end of the experiment (cell number)

**Metformin Treatment:**
**Decreased cell proliferation without cytotoxicity**




**Bioluminescent LDH Release Assay**




## 9. Summary

**The bioluminescent metabolite assays are sensitive assays useful for cancer and immune cell metabolism research**

- Bioluminescent NAD(P)H detection is a versatile technology that can be adapted for measuring several metabolites
- Metabolites in a variety of sample types can be measured, including: cell lysates, culture media, tissues and plasma/sera
- Only small amounts of sample are needed due to high assay sensitivity and multiple metabolites can be measured from the same sample
- An individual population of cells can be monitored over time by removing small volumes of culture medium, preserving the cells for further studies
- For cell lysates, lysis can be done directly in the well, simplifying sample preparation
- Alterations in metabolic pathways can be detected by measuring key metabolites such as glucose, lactate, glutamine and glutamate
- T cell activation can be easily followed using increased lactate production as a marker for the metabolic switch that occurs upon activation



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