Bioluminescent Platform for Cellular Energy Metabolism Studies

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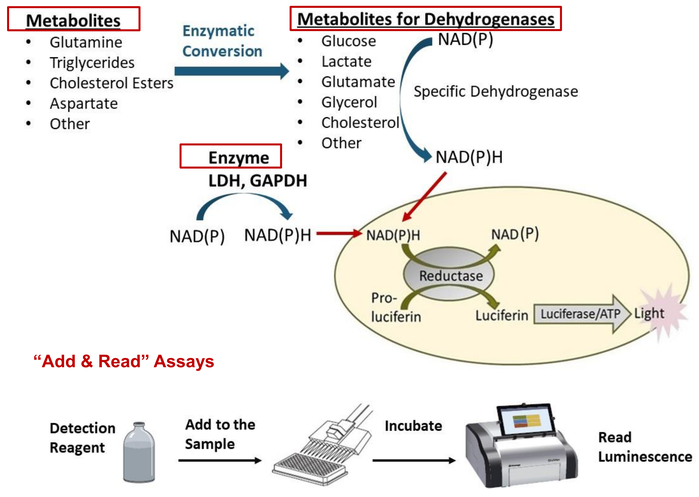
1. Introduction

Energy uptake and utilization in eukaryotic cells is a dynamic process regulated by a series of interacting metabolic networks. Interrogation of this complex network relies on rapid, sensitive approaches that do not require extensive sample handling, are easily adaptable to 384-, 1536-well format and therefore can facilitate novel compound screening and evaluation. Utilizing a novel proluciferin substrate that in the presence of NAD(P)H is converted to luciferin and light production by luciferase, we developed a core technology for measuring key energy co-factors (NAD(P)/NAD(P)H) and extended it to multiple metabolite (glucose, lactate, glutamine, glutamate, glycerol, triglycerides) or enzyme activity assays. Here we demonstrate applicability of those assays to various formats (96-, 384- and 1536-well plates) and samples (2D, 3D cultures) using multiple automation platforms, including collecting the samples at different time points and performing multiple assays for the same sample using the Labcyte Echo acoustic dispenser.

2. Platform Overview: Based on NAD(P)H Detection

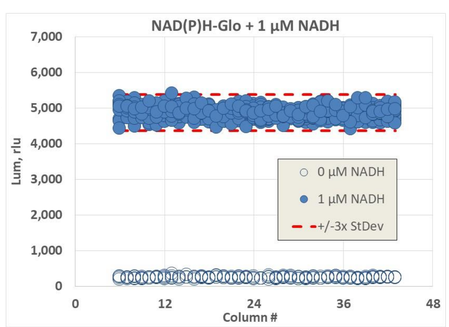


3. Applicability to Miniaturization and Automation

NADH detection in 1536-well plates (4µl Sample + 4µl Detection Reagent):

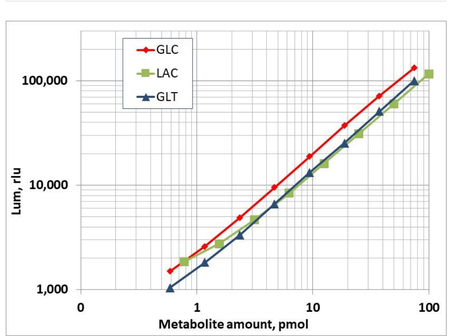
S/B = 20

Z' = 0.832



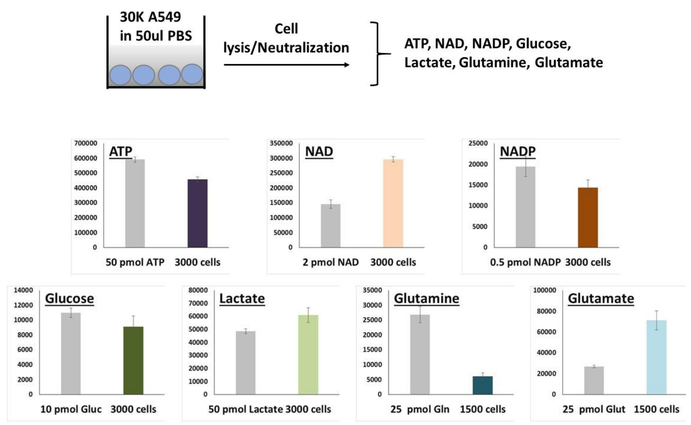
Glucose, Lactate and Glutamate titration curves in 1536-well plates:

60nl Sample (added to 4µl Buffer) 4µl Detection Reagent



4. Multiple Metabolites from Small Amount of Cells with Minimal Sample Preparation

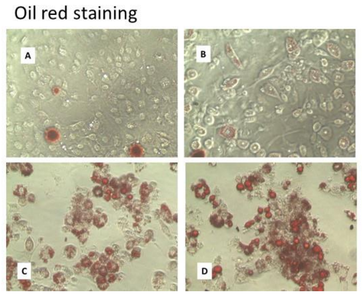
Multiple Metabolites were measured from one well of cells in 96-well plate



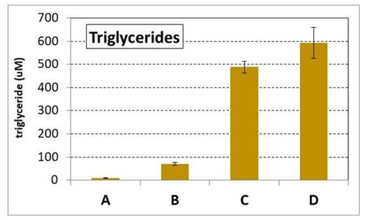
Metabolites	ATP	NAD	NADP	Glucose	Lactate	Glutamine	Glutamate
fmol/cell	6	0.8	0.05	0.8	13.6	3.8	44

Lipid Accumulation During Adipocyte Differentiation

Oil red staining



Triglyceride accumulation using bioluminescent assay



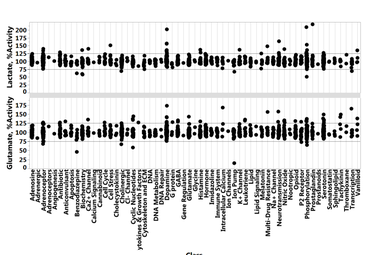
Panel A: fibroblasts;
Panel B: differentiation stage 1;
Panel C: differentiation stage 2;
Panel D: mature adipocytes

5. HTS Screening for Glycolysis and Glutaminolysis Inhibitors

Screening LOPAC 1280 Library with SKOV-3 Cells in 384-LV plates

	Step	Volume or time	Details
1	Library Compounds		5nl of 10mM compounds in DMSO; each compound in quadruplicate wells in 384-well LV plates
2	Cells	6µl	1000 SKOV-3 cells/well; cell suspension in DMEM containing 1X real-time viability reagents
3	Control	6µl	DMEM with 0.1% DMSO
4	Incubation	15min	Room temperature
5	Media	2µl	DMEM containing 20mM Glucose and 8mM Glutamine
6	Incubation	1hr	37°C, 5% CO_2
7	Viability read-out		Luminescence
8	Stop	1µl	0.6N HCl/0.1% DTAB; room temperature
9	Detection reaction	9µl	Lactate or glutamate detection reagent containing 1M Trizma
10	Incubation	90min	Room temperature
11	Lactate or Glutamate read-out		Luminescence

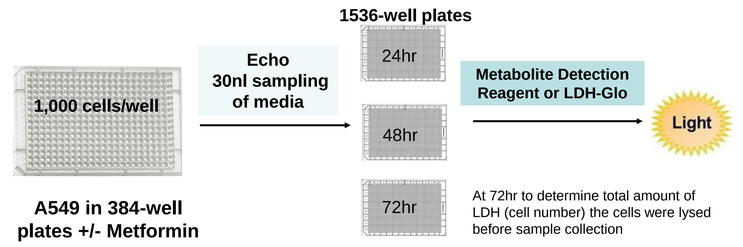
- Compounds decreasing viability by >75% were eliminated
- Lactate and Glutamate values were normalized to viability readings



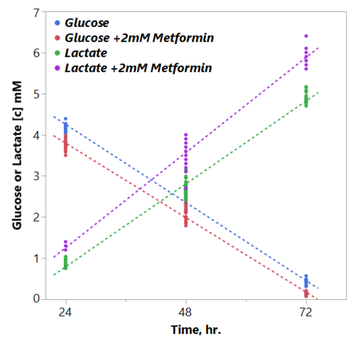
Leippe, D *et al.* (2017) *SLAS Discovery*, Vol.22, 366-377

6. Following Metabolite Consumption and Secretion During Cell Treatment

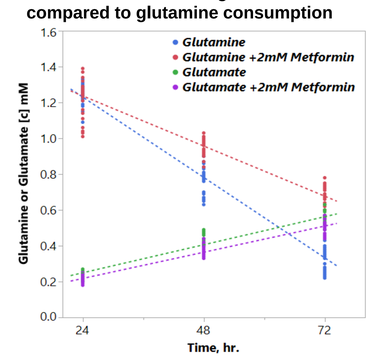
Using Small Volume Acoustic Dispensing with Metabolite and LDH-Glo Assays



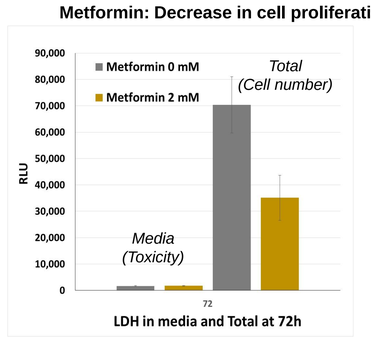
Metformin: Increase in aerobic glycolysis



Metformin: Increase in glutamate secretion compared to glutamine consumption



Metformin: Decrease in cell proliferation



- Using acoustic dispensing method, nanoliter media samples can be collected at different time points without affecting the total volume of cell culture.
- The sensitivity of metabolite and LDH-Glo assays allows two major energy metabolism pathways, glycolysis and glutaminolysis, to be assessed from the same cell population and compared to changes in cell viability/cell growth.

7. Conclusions

Bioluminescent energy metabolism platform is based on the core NAD(P)H detection technology:

- Provides sensitive detection (1-5 pmol/sam**ple)** with broad linear range (0.1-100µM) and wide dynamic range (max S/B >100 fold)
- Amendable to miniaturization with Z'>0.8 in 1536-well plates

The technology can be extended for multiple metabolite detection in different sample types:

- Glucose, Lactate, Glutamine, Glutamate, Glycerol, Triglyceride, Cholesterol, Cholesterol esters
- Cell medium and cells in culture, 3D models, tissues

Advantages:

- Multiple metabolites can be detected from the same sample
- Wide linear range provides flexibility when measuring metabolites over wide concentration range
- Optimized rapid in well cell lysis compatible with homogeneous format makes it suitable for HTS screening
- Multiplexing with viability/toxicity readouts provides normalization and allows separating effects on metabolism from global effects on cell health

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