Cell Metabolism HTS Assays: Glucose, Lactate, Glutamine, and Glutamate Detection

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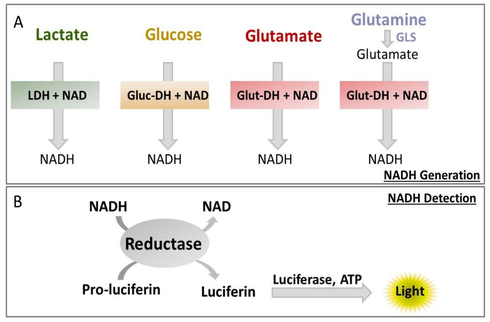
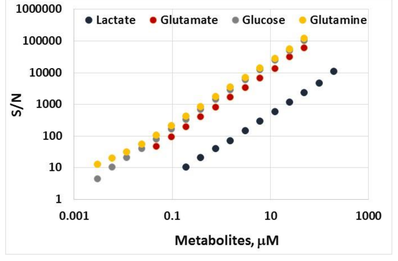
1. Introduction

Monitoring Cellular Energy Metabolism: Disturbances of the balances in cellular metabolism are associated with various maladies including cancer. Cancer cell metabolism is a complex, dynamic network of regulated pathways including glycolysis and glutaminolysis. Here we describe bioluminescent glucose, lactate, glutamine, and glutamate detection assays that are well-suited for high-throughput analysis. The assays described here are suitable for measuring extracellular and intracellular metabolites. In addition, cell metabolism assays can be multiplexed with a real-time cell viability assay.



2. Assay Performance



Example assay set up in 96-well plate

50µl Metabolite in PBS

+

50µl Metabolite Detection Reagent

Incubate 1h

Read Luminescence

Assay	LOD	Linearity	S/B max
Lactate	100nM (5pmol/50µl)	Up to 200µM	> 240
Glucose	5nM (0.25pmol/50µl)	Up to 50µM	>1000
Glutamine	5nM (0.25pmol/50µl)	Up to 50µM	> 1000
Glutamate	5nM (0.25pmol/50µl)	Up to 50µM	> 500

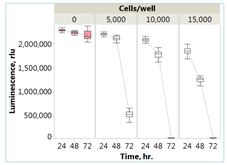
3. Extracellular HTS Assay Screening Protocols

Detection of extracellular metabolites in medium using Standard Volume 384 well plates

	Step	Volume or time	Details
1	Cells	80µl	SKOV-3 or OVCAR-3 cells in DMEM
2	Reaction Start	10µl	5mM Glucose, 2mM Glutamine and 10% dialyzed FBS
3	Incubation	Xhr	37°C, 5%CO_2
4	Sampling	4µl	at 24, 48, 72hr medium transferred into 96-well plates containing 96µl PBS/well
5	Storage	X days	-20ºC
6	Metabolite analysis	9µl or 4.5µl	9µl sample for lactate detection; 4.5µl sample + 4.5µl buffer for glucose, glutamine and glutamate detection
7	Detection reaction	9µl	Lactate-Glo™ or Glutamate-Glo™ System
8	Incubation	90min	Room temperature
9	Metabolite read-out		Luminescence

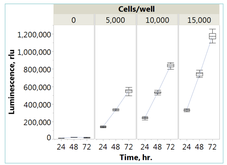
4. Extracellular HTS Assay Evaluations

Glucose Consumed



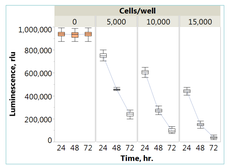
Glucose-Glo Z' values			
	Reaction Time		
Cells/well	24 hr.	48 hr.	72 hr.
5,000	-	-	0.64
10,000	-	0.31	0.83
15,000	0.42	0.75	0.83

Lactate Secreted



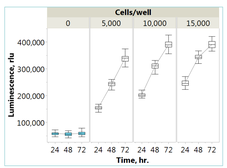
Lactate-Glo Z' values			
	Reaction Time		
Cells/well	24 hr.	48 hr.	72 hr.
5,000	0.85	0.91	0.86
10,000	0.87	0.92	0.91
15,000	0.91	0.81	0.88

Glutamine Consumed



Glutamine-Glo Z' values			
	Reaction Time		
Cells/well	24 hr.	48 hr.	72 hr.
5,000	0.09	0.60	0.60
10,000	0.54	0.70	0.70
15,000	0.70	0.75	0.75

Glutamate Secreted



Glutamate-Glo Z' values			
	Reaction Time		
Cells/well	24 hr.	48 hr.	72 hr.
5,000	0.47	0.77	0.66
10,000	0.65	0.80	0.71
15,000	0.67	0.83	0.73

All: SKOV-3 cells

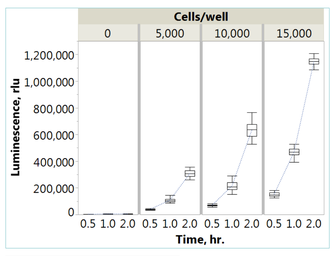
5. Homogeneous HTS Assay Screening Protocols

Homogeneous Lactate and Glutamate detection using Low Volume 384 well plates

	Step	Volume or time	Details
1	Cells	4µl	OVCAR-3 or SKOV-3 cells in DMEM (+ 1X RealTime-Glo™ Viability Assay reagents)
2	Reaction Start	2µl	15mM Glucose, 6mM Glutamine
3	Incubation	Xhr	37°C, 5%CO_2
4	Reaction time	0.5, 1.0 or 2.0hr	All plate content
5	Cell viability		RealTime-Glo™ Viability Assay read
6	Reaction Stop	1µl	0.6N HCl / 0.1% DTAB; room temperature
7	Detection reaction	9µl	Lactate- or Glutamate-Glo™ System reagents containing 111mM Trizma
8	Incubation	60min	Room temperature
9	Metabolite read-out		Luminescence

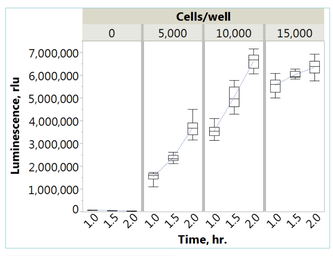
6. Homogeneous Assay Evaluations

Lactate Accumulation



Lactate-Glo Z' values			
	Reaction Time		
Cells/well	0.5 hr.	1.0 hr.	2.0 hr.
5,000	0.75	0.53	0.74
10,000	0.70	0.61	0.71
15,000	0.69	0.80	0.92

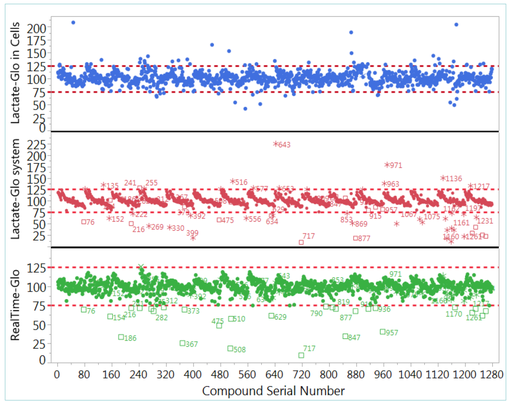
Glutamate Accumulation



Glutamate-Glo Z' values			
	Reaction Time		
Cells/well	1.0 hr.	1.5 hr.	2.0 hr.
5,000	0.63	0.81	0.72
10,000	0.80	0.79	0.79
15,000	0.82	0.92	0.84

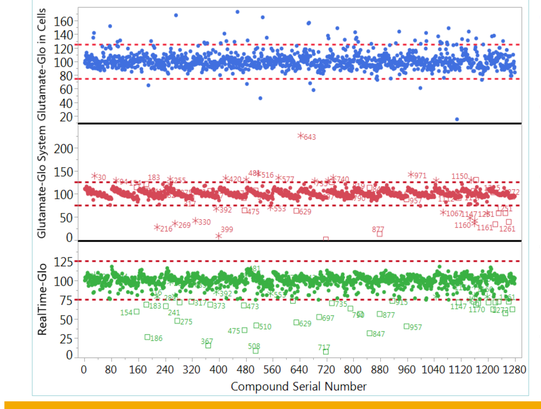
All: SKOV-3 cells, Low Volume 384-well plates

7. Lactate-Glo™ Assay vs. LOPAC Library Screens and Multiplexing



- **Identified LOPAC library compounds effecting Lactate metabolism in the SKOV cells.**
- **Lactate-Glo™ System** counter-screen using 50 µM lactate.
- **RealTime-Glo™ Assay** SKOV cell viability assay normalization-screen.
- Numbered Squares □ are LOPAC library compounds affecting cell health (RealTime-Glo™ Assay).
- Numbered Stars ✳ are compounds affecting Lactate-Glo™ System.

8. Glutamate-Glo™ Assay vs. LOPAC Library Screens and Multiplexing



- **Identified LOPAC library compounds effecting Glutamate metabolism in the SKOV cells.**
- **Glutamate-Glo™ System** counter-screen using 50µM glutamate.
- **RealTime-Glo™ Assay** SKOV cell viability assay normalization-screen.
- Numbered Squares □ are LOPAC library compounds affecting cell health (RealTime-Glo™ Assay).
- Numbered Stars ✳ are compounds affecting Glutamate-Glo™ System.

9. Conclusions

Benefits of the new cellular Energy metabolism assays

- Wider assay window and broad linearity compared to colorimetric and fluorometric assays
- Detect lactate, glucose, glutamate, or glutamine from cell culture media, cells, or tissue, with minimal sample preparation
- No sample deproteinization, centrifugation, or spin columns required
- Inactivation and neutralization solutions for easy sample preparation
- Compatible with standard and low volume 384 well formats for assay miniaturization
- Assays are suited for measuring both extracellular and intracellular (homogeneous) metabolites

Multiplexing screening benefits

- Specific, robust, and sensitive
- Live cell assay in real-time with kinetic or end point measurements
- Compatible with standard and low volume 384-well formats for assay miniaturization

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