1323-D

Implementation of Acoustic Dispensing for Kinetic Monitoring of Glycolysis and Glutaminolysis.

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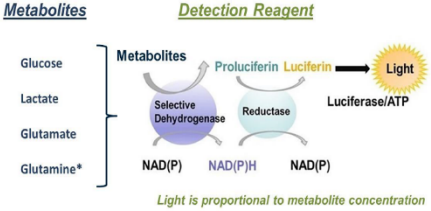
1. Abstract

Glycolysis and glutaminolysis are two major energy metabolism pathways required for rapid cell proliferation. In the cancer microenvironment, cancer cells and activated T lymphocytes exhibit similar metabolic profiles as they compete for the same nutrients. Understanding and targeting these metabolic pathways, therefore, can provide valuable information on the function of those cells and lead to more effective cancer treatment strategies.

Contamination and carryover-free acoustic liquid handling technology has been widely implemented into high-throughput applications. Here we evaluated the novel application of small volume acoustic sampling for measuring cell metabolism using bioluminescent metabolite assays. Normal usage of these high sensitivity metabolism assays requires sample pre-dilution. In contrast, use of the Labcyte Echo® Liquid Handler eliminates this pre-dilution step as it is able to dynamically adjust to differing fluid properties and is able to transfer from any well to any well.

This type of rapid and small volume sampling has minimal impact on the cell growth conditions and in combination with bioluminescent metabolite assays that are well suited for miniaturization provides a unique approach for easy and fast repetitive cell metabolism monitoring.

2. Bioluminescence Metabolite Assays

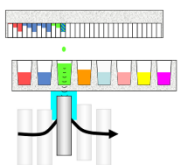


*Glutamine detection requires a two step protocol: glutamine is converted to glutamate before adding glutamate detection reagent

Leippe, D. et al. SLAS Discovery. 2017, 22, 366-377

3. Labcyte Echo

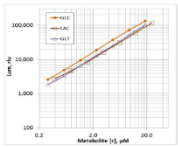
The Labcyte Echo® 500 series liquid handlers revolutionize liquid transfer by using acoustic energy to eject fluids. Transfer with Echo liquid handlers is completely touchless — no tips or nozzles, and no material contacts the sample as it moves from source to destination. The elimination of tips when using the Echo liquid handler provides additional cost savings and eliminates waste, carry-over effects, and cross-contamination. The Echo 555 liquid handler can transfer in 2.5 nL increments to allow miniaturization with accuracy and precision.



Echo 555 liquid handlers have a transducer that emits low energy sound waves to eject 2.5 nL droplets from a source plate to an inverted destination plate above. Droplets are retained in the destination plate by electrostatics and surface tension.

4. Experimental Design and Validation

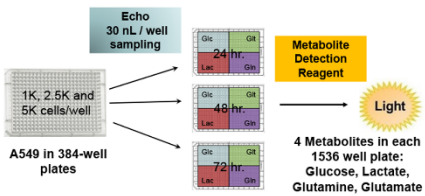
Experimental design and validation in 1536-well plates using metabolite standards (glucose, lactate and glutamine)



	Step	Volume	Details
1	Prepare Metabolite Standards	60 µl	Prepare in medium in 384-well plates
2	Prepare Sample collection plates	4.0 µl	Pre-dispense PBS into 1536-well plates
3	Collect samples	30 nl	Use Echo® to collect. Samples can be analyzed immediately or stored at -20C
4	Detect Metabolites	4.0 µl	Add appropriate detection reagent and read luminescence after 1hr incubation at room temperature

5. Measuring Metabolites in Culture Medium

To detect changes in metabolite concentration during cell growth, media samples are collected at different time points, frozen and analyzed at the end of experiment

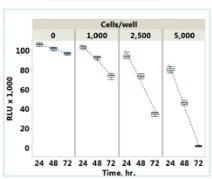


6. Glycolysis: Measuring Glucose Consumption and Lactate Secretion

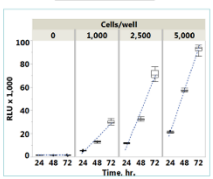
A549 cancer cells were cultured in DMEM with 5mM glucose, 2mM glutamine and 4% dialyzed FBS:

- Rapid glucose consumption with high levels of lactate production – consistent with glycolytic phenotype of cancer cells

Glucose Consumption



Lactate Secretion



Glucose-Glo 2' values

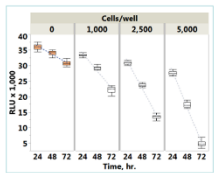
Cells/well	Reaction Time 24 hr.	48 hr.	72 hr.
1,000	0.97	0.96	0.93
2,500	0.95	0.95	0.88
5,000	0.93	0.92	--

Lactate-Glo 2' values

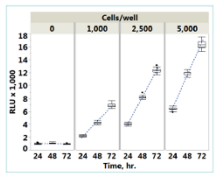
Cells/well	Reaction Time 24 hr.	48 hr.	72 hr.
1,000	0.81	0.86	0.86
2,500	0.90	0.91	0.84
5,000	0.92	0.95	0.90

7. Glutaminolysis: Measuring Glutamine Consumption and Glutamate Secretion

Glutamine Consumption



Glutamate Secretion



Glutamine-Glo 2' values

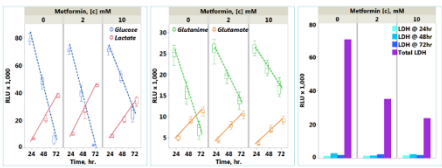
Cells/well	Reaction Time 24 hr.	48 hr.	72 hr.
1,000	0.72	0.82	0.86
2,500	0.85	0.90	0.90
5,000	0.86	0.92	0.88

Glutamate-Glo 2' values

Cells/well	Reaction Time 24 hr.	48 hr.	72 hr.
1,000	0.96	0.96	0.92
2,500	0.96	0.95	0.79
5,000	0.94	0.90	0.85

8. Metformin: Inhibition of Glycolysis and Glutaminolysis with Decrease in Cell Proliferation

Affect of metformin, an inhibitor of the mitochondrial respiratory-chain complex 1, on glucose, lactate, glutamine, glutamate metabolism and cell growth



Metformin treatment:

- No effect of cell toxicity - no change in LDH release into the medium
- Decrease in cell numbers - decrease in total LDH (LDH after cell lysis)
- Increase in aerobic glycolysis - No or slight decrease in glucose consumption with no significant change in lactate
- Decrease in glutamine consumption with no significant changes in glutamate secretion

9. Conclusions

Bioluminescent Cellular Energy Metabolism Assays

- Wider assay window, broader linearity and improved sensitivity as compared to colorimetric and fluorometric assays
- Amenability to automation with robust performance in 384-, 1536-well plates

Acoustic Dispensing

- Fast and accurate cell culture medium sampling at different time points of cell growth or treatment
- Nanoliter sampling volumes do not change the volume of total cell culture – multiple samples can be collected from the same well

Implementation of Acoustic Dispensing with Bioluminescent Metabolite Assays

- Minimal sample handling – samples can be collected at different time points and analyzed directly in 1536-well plates
- Changes in key metabolic pathways, glycolysis and glutaminolysis, can be analyzed rapidly in high-throughput format

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