HOW HEALTHY ARE YOUR CELLS?

Tools for Discovering the Truth

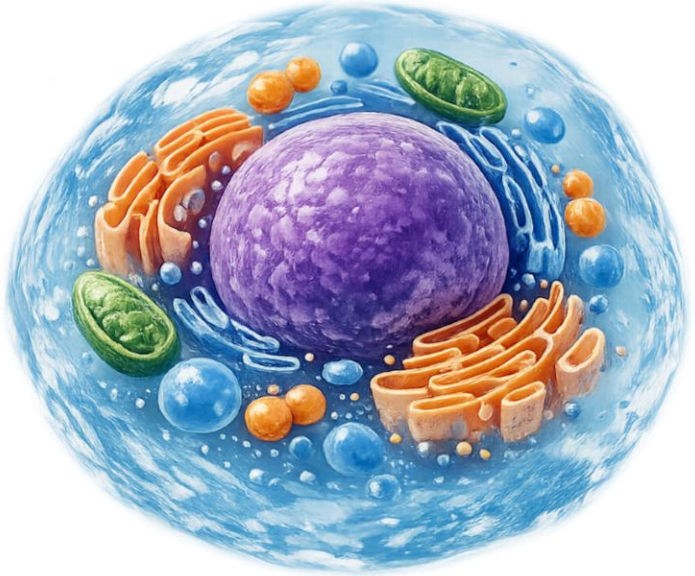
Mgr. Lukáš Išler

&

Kerem Yildirim (Promega)

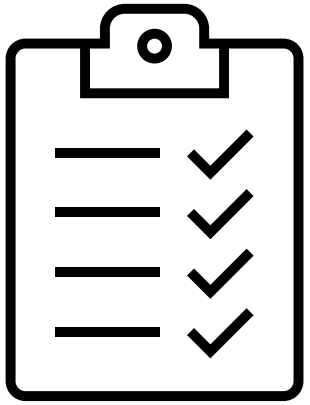
Sylvia de Bruin (Molecular Devices)

EASTPORT LIFESCIENCE



Generated by OpenAI

Program



Introduction to cell-based assays, NanoLuc technologies (Kerem Yildirim)
Coffee break, Q&A
Metabolism changes in cancer cells; label-free analysis of cells (Lukáš Išler)
High-content imaging (Sylvia de Bruin)
Final thoughts and discussion

About East Port

About us

- Over **25 years** supporting life science research in the Czech Republic (+ Slovakia)
- More than **15 partnerships** with leading biotechnology suppliers
- Team with scientific background

Company Overview

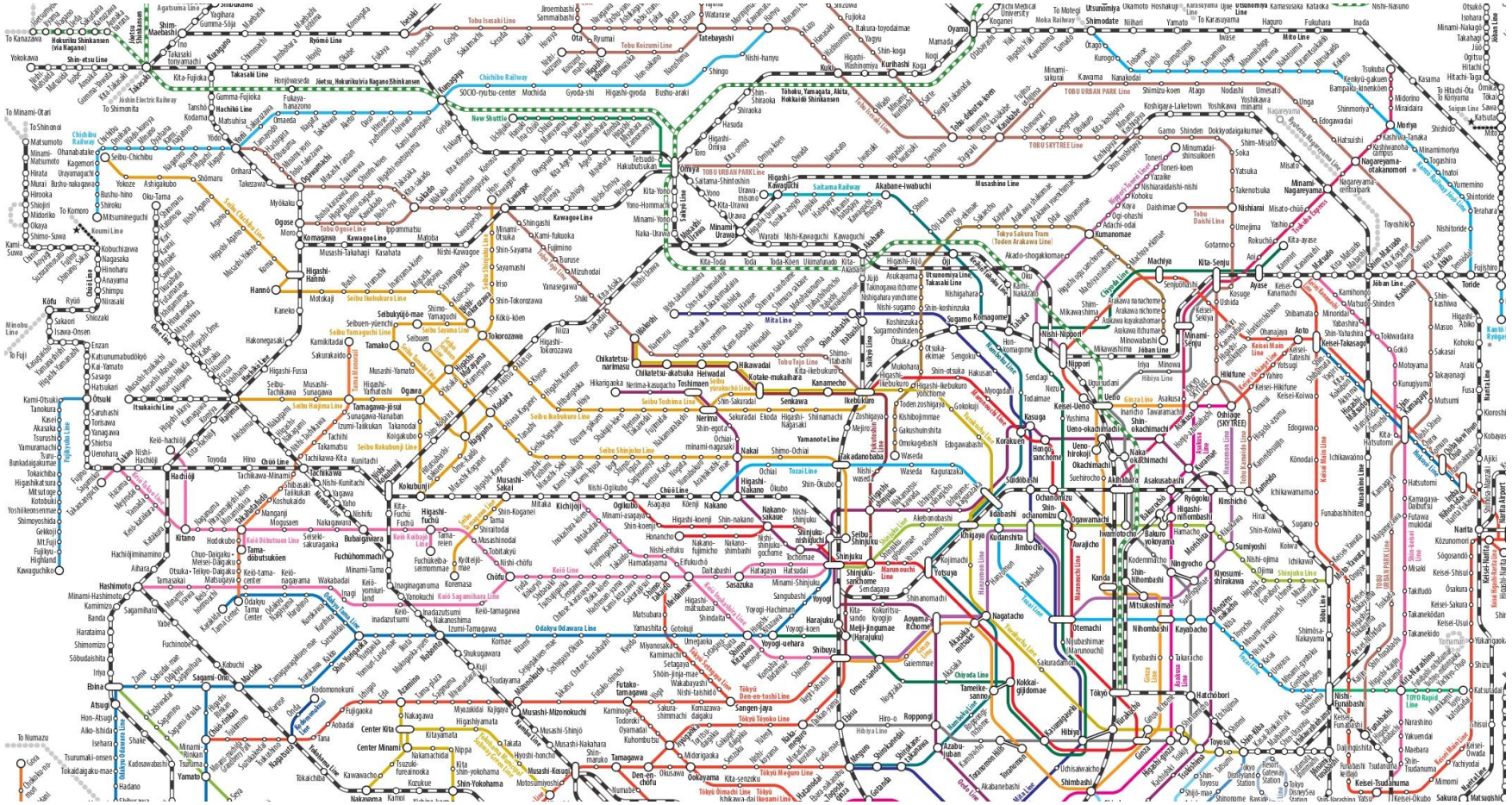
We Provide

- Innovative biological reagents and instrumentation
- Scientific application support
- Technical service

Covered Product Areas

- Molecular and cell biology
- Instruments
- Consumables
- In vitro fertilization

Metabolism



Metabolism

https://photos.smugmug.com/Transit-Maps/Japan/i-KNVpKMH/0/c5348fa8/O/tokyo-suica-pasmo-network.jpg

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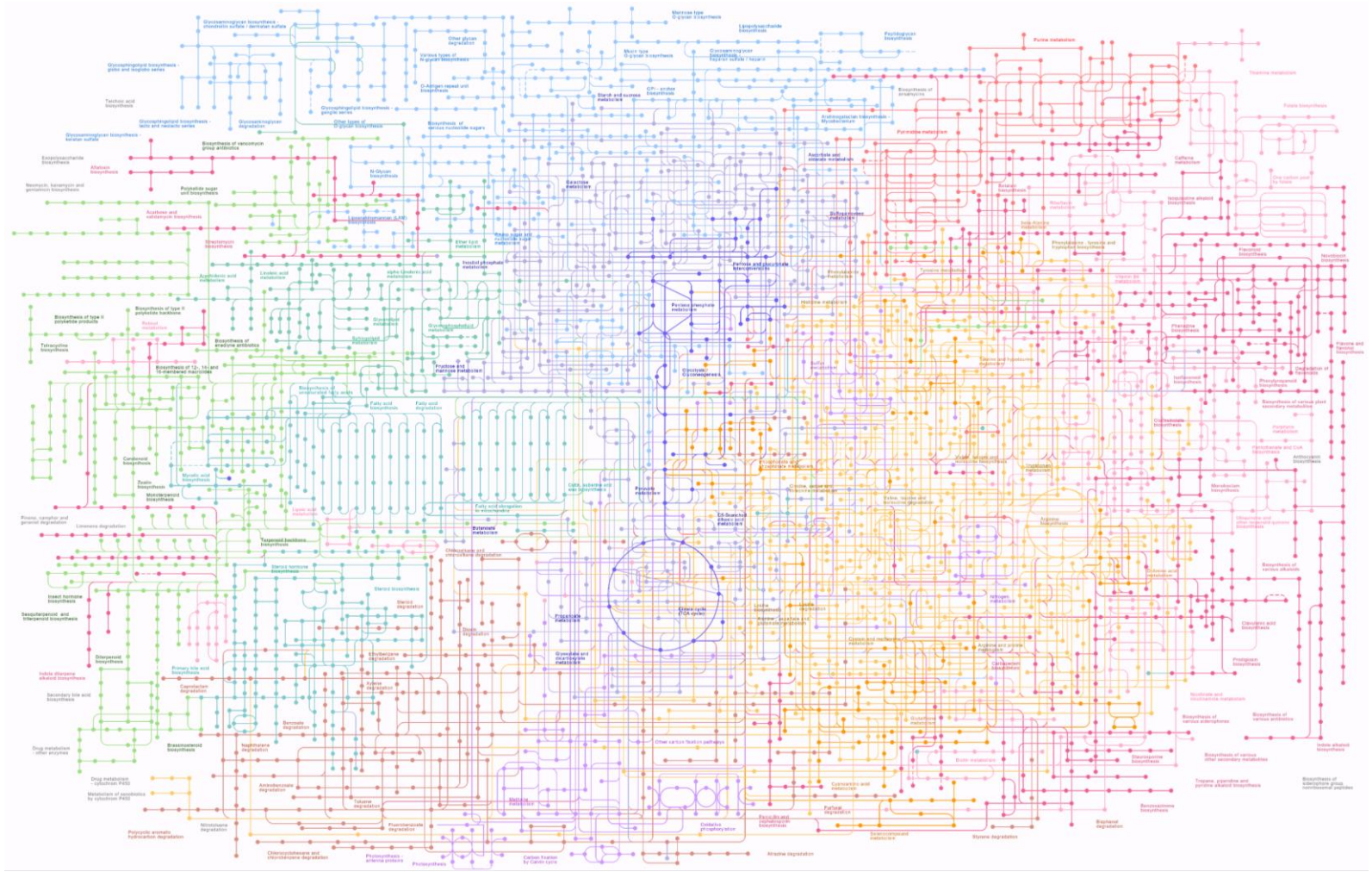
Metabolism



https://www.genome.jp/kegg/pathway.html#metabolism

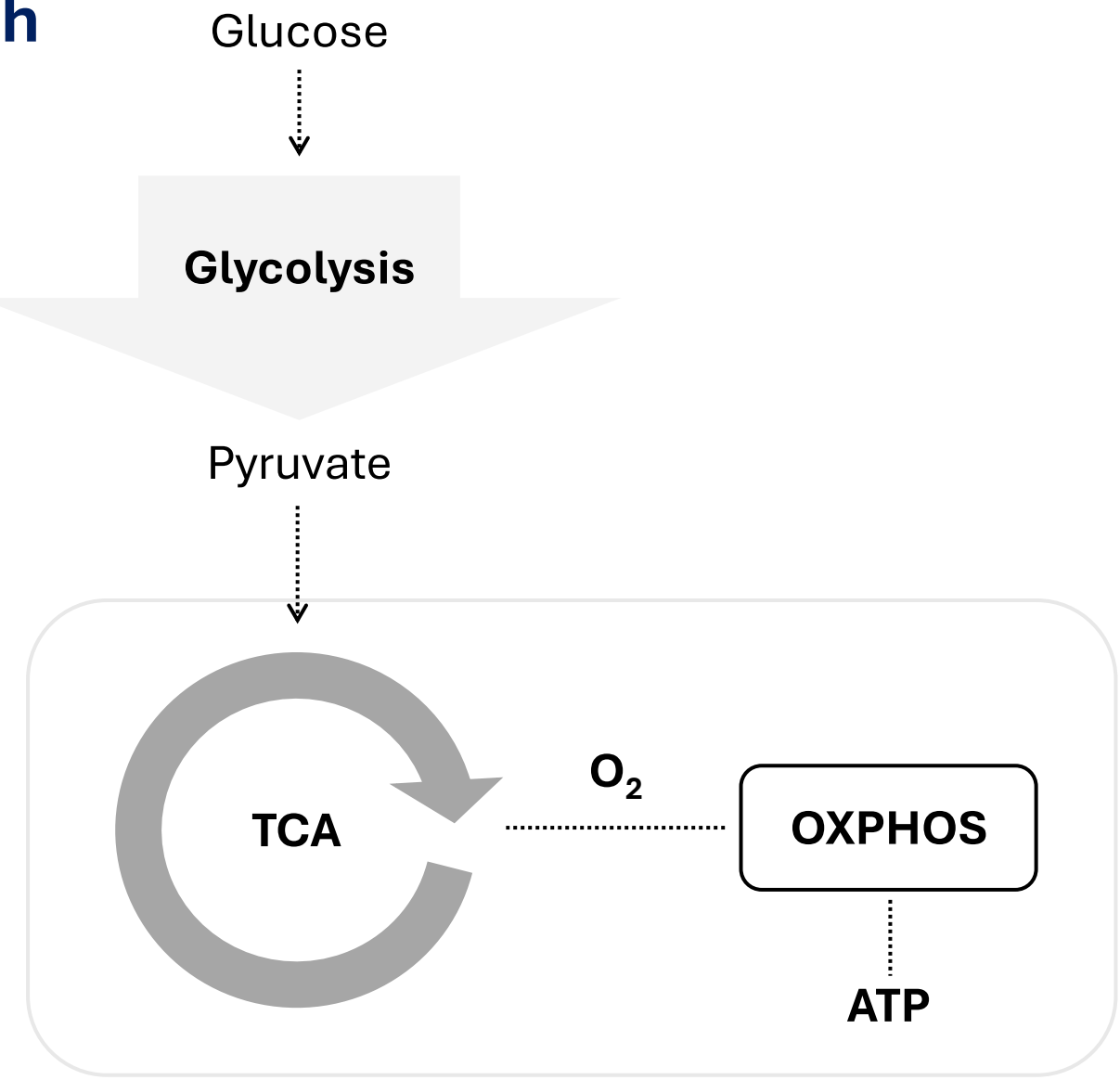
EASTPORT LIFESCIENCE

Major Changes for Tumor to Growth

- Energy Demand
- Building blocks
- Redox Balance & ROS
- Hypoxia Adaptation

Healthy Cells Power Themselves with

- Combination of **glycolysis** & **OXPHOS**
- Efficient ATP production in **mitochondria**
- Metabolism tightly regulated by growth signals and nutrient availability



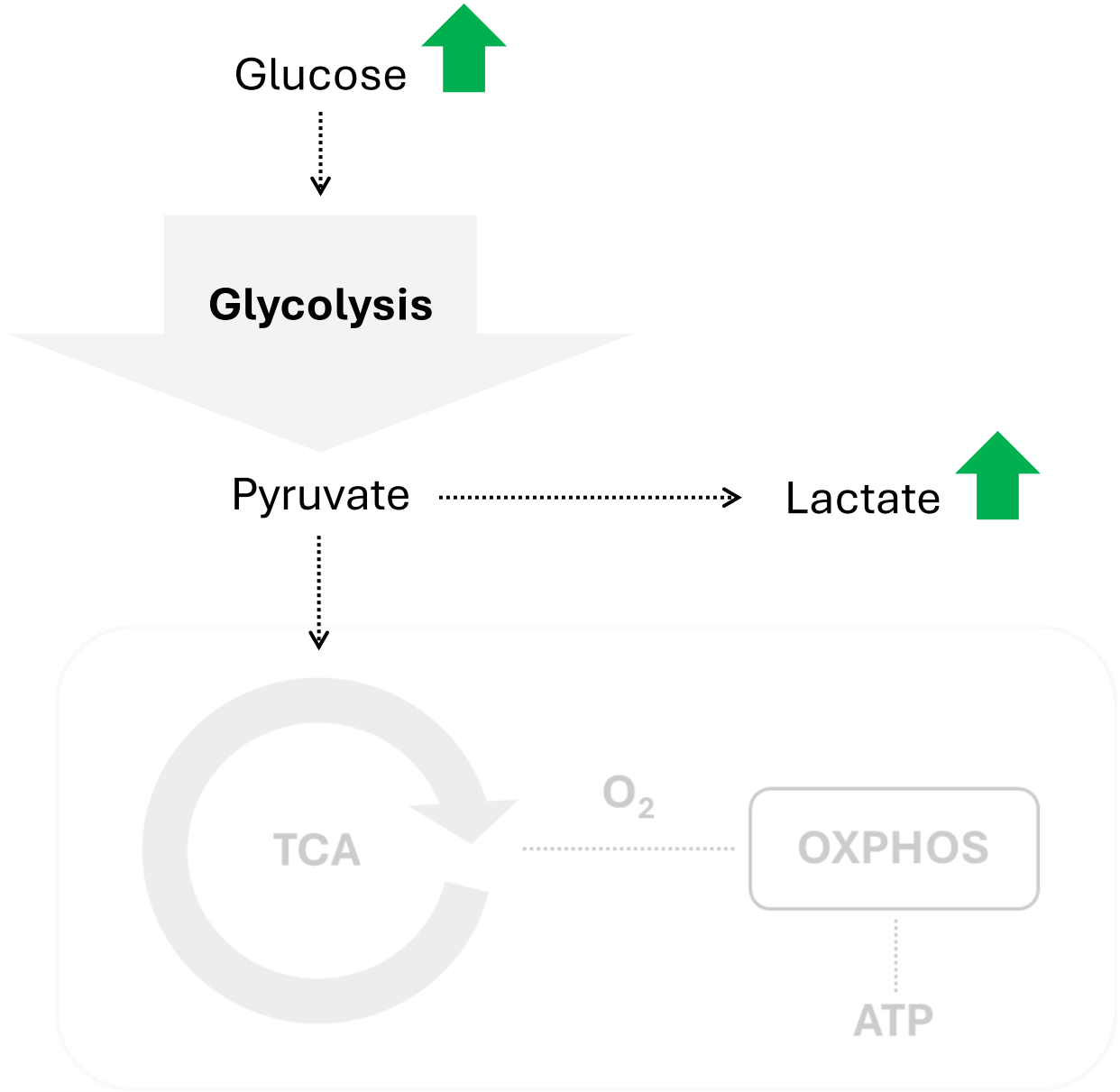
Key Takeaway: Healthy cells prioritize energy efficiency. Mitochondria are the central hub for producing ATP, regulating redox balance, and coordinating biosynthesis.

TCA = tricarboxylic acid cycle/Krebs cycle; OXPHOS = oxidative phosphorylation

Reprogramming in Cancer Cells

- Preference for **aerobic glycolysis**
- Even in **normoxia**, glycolysis is prefered
- **High** glucose uptake, **high** lactate production

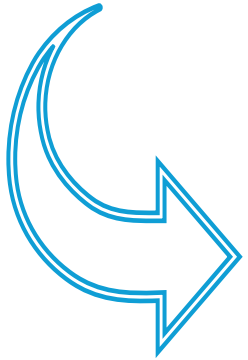
Warburg effect



TCA = tricarboxylic acid cycle/Krebs cycle; OXPHOS = oxidative phosphorylation

Paradox of Warburg effect

- **Lower ATP yield** from glycolysis → 1 Gluc = **+ 2 ATP** vs **30-32** ATP from OXPHOS
- Why the preference then?



Speed of ATP production

Glycolysis is 10–100× faster than OXPHOS.

Biosynthetic precursors

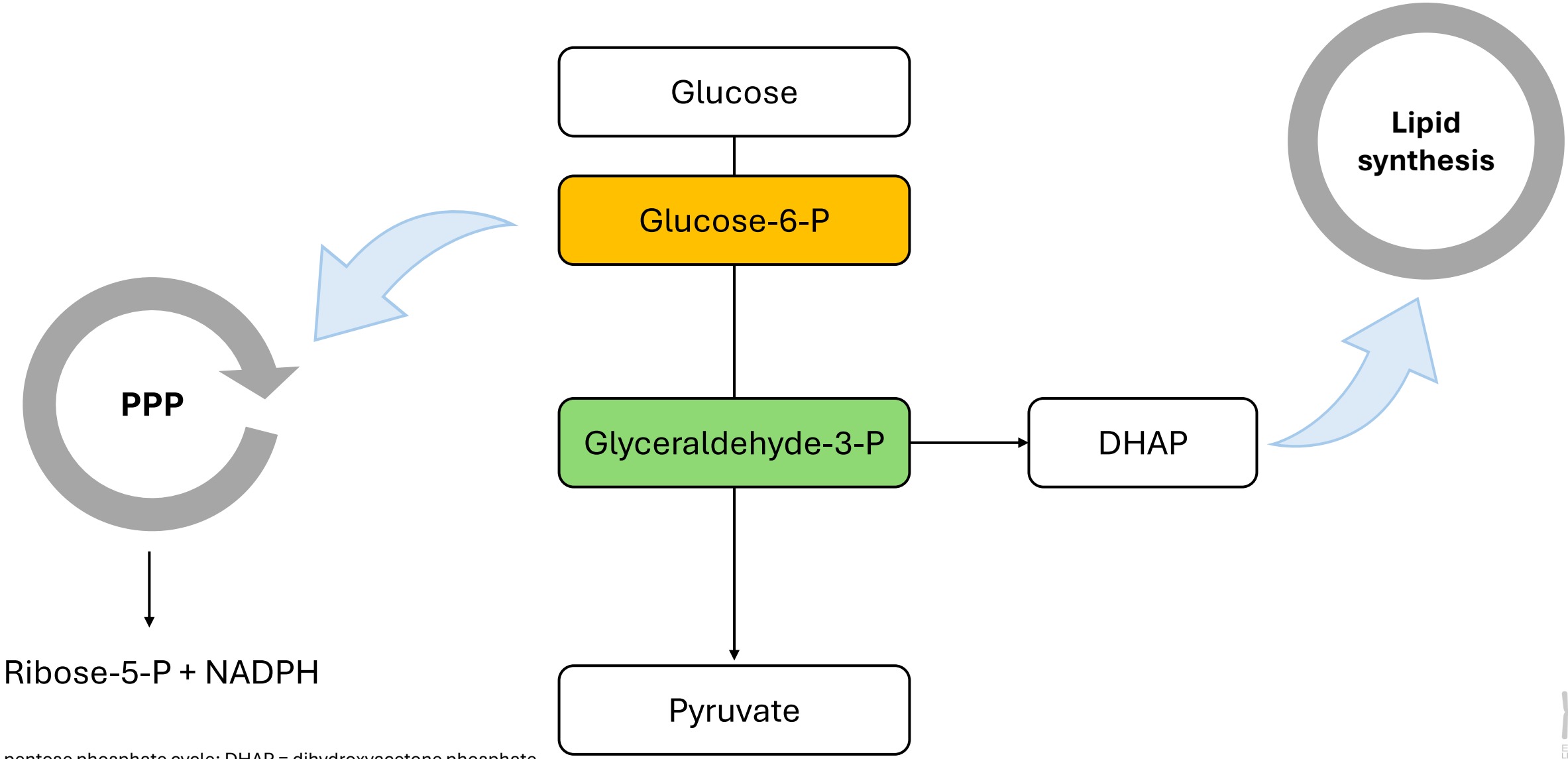
Glycolytic intermediates feed nucleotide, amino acid, and lipid synthesis pathways.

Acidification of enviroment

Lactate creates conditions for promoting invasion and immune evasion.

OXPHOS = oxidative phosphorylation

Glycolysis: Intermediates for other pathways

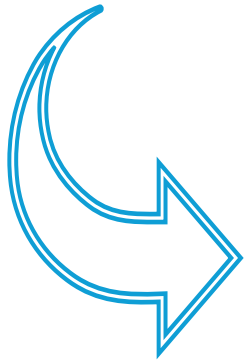


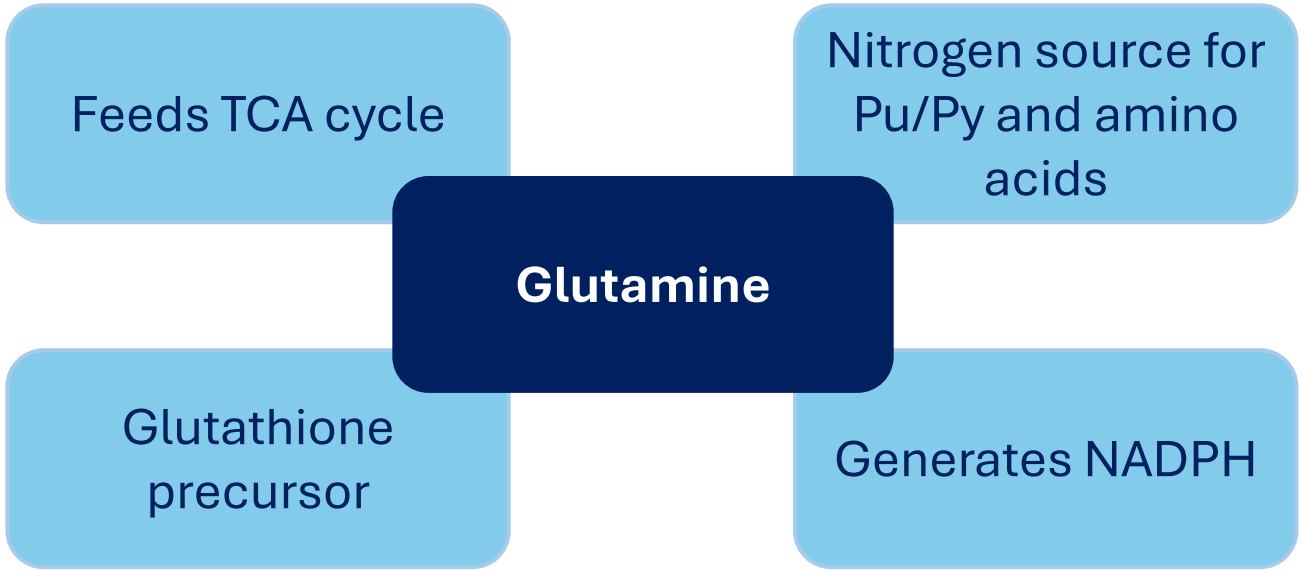
PPP = pentose phosphate cycle; DHAP = dihydroxyacetone phosphate

Glutamine: The Second Fuel

- Why to be glutamine addicted?

Generated by Gemini



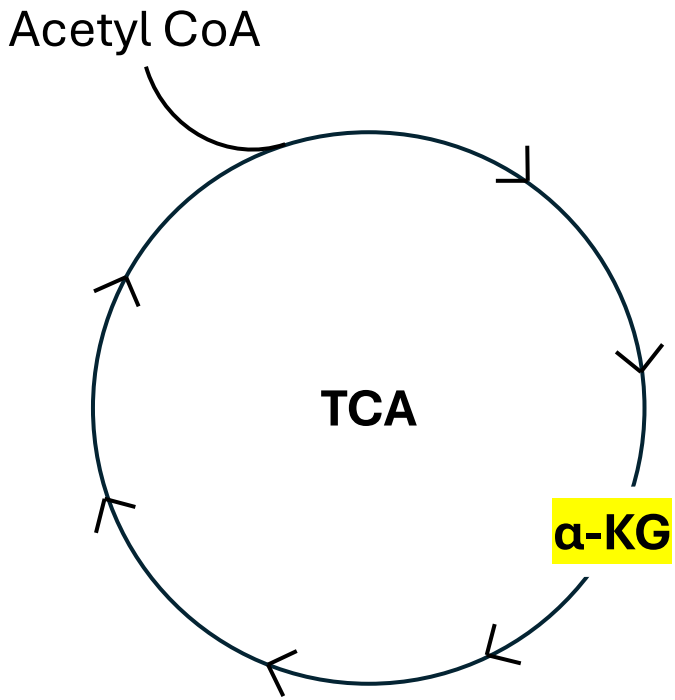


TCA = tricarboxylic acid cycle/Krebs cycle

From Glutamine to α-ketoglutarate to TCA Cycle

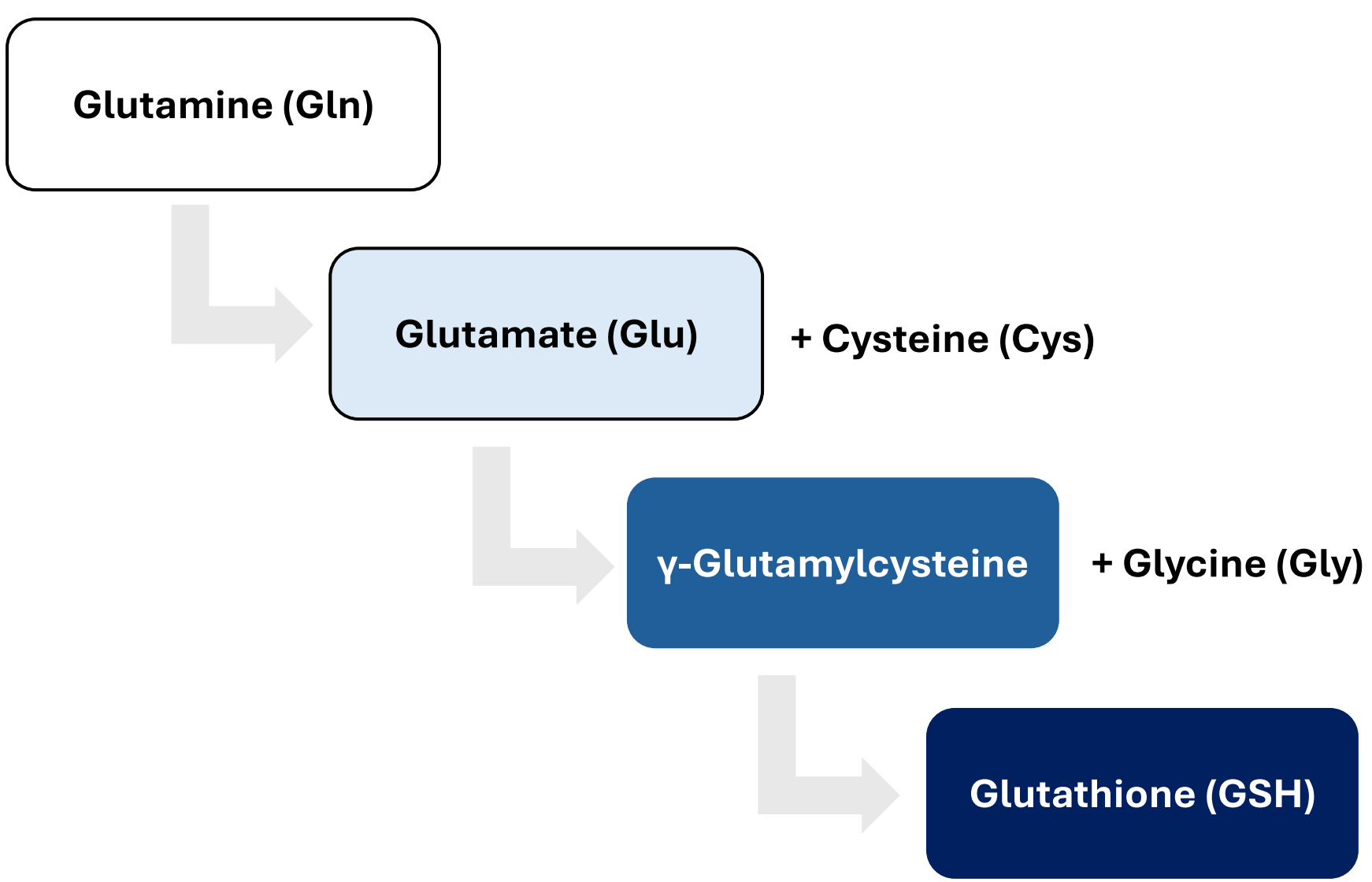
$$\textbf{Glutamine} \xrightarrow{\text{Glutaminase}} \textbf{Glutamate} + NH_4^+$$

$$\textbf{Glutamate} \xrightarrow{\text{Glutamate Dehydrogenase}} \alpha\textbf{-Ketoglutarate} + NH_4^+ + NADH$$



TCA = tricarboxylic acid cycle/Krebs cycle; CoA = coenzyme A

Glutamine: Glutathione precursor

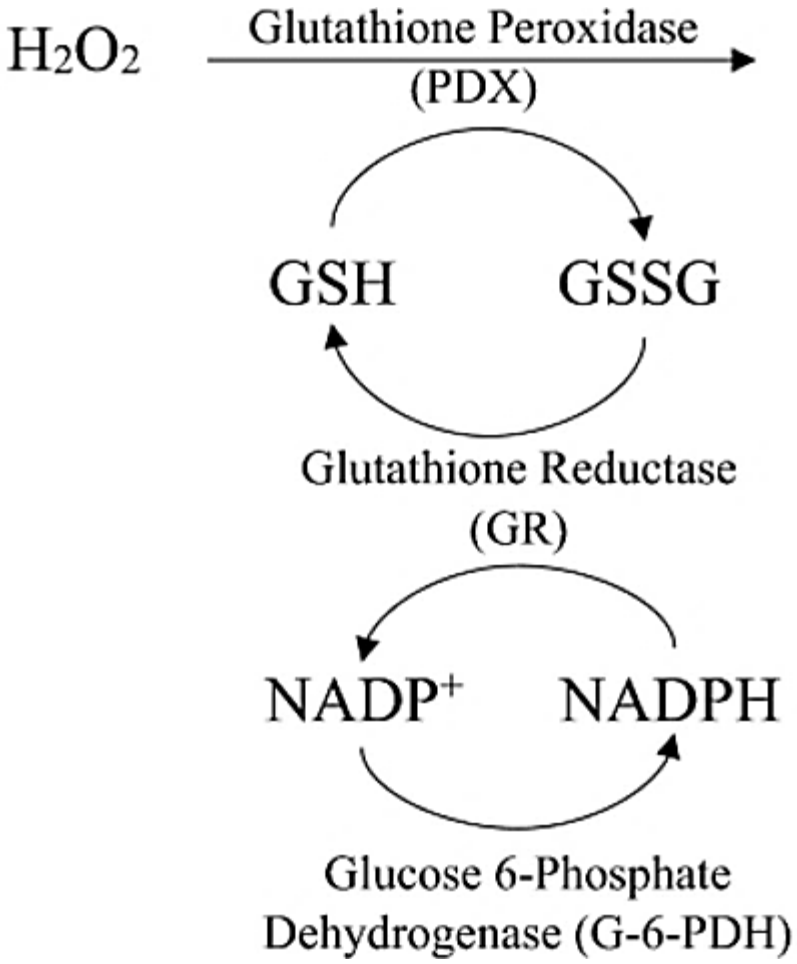


GSH = Glu-Cys-Gly

Glutathione: Master of Antioxidants

- Increased **ROS** production in tumor cells
- ROS promotes **growth** and **angiogenesis**
- Incresed ROS production needs to compensated

Increased **Glutathione** and **NADPH** production

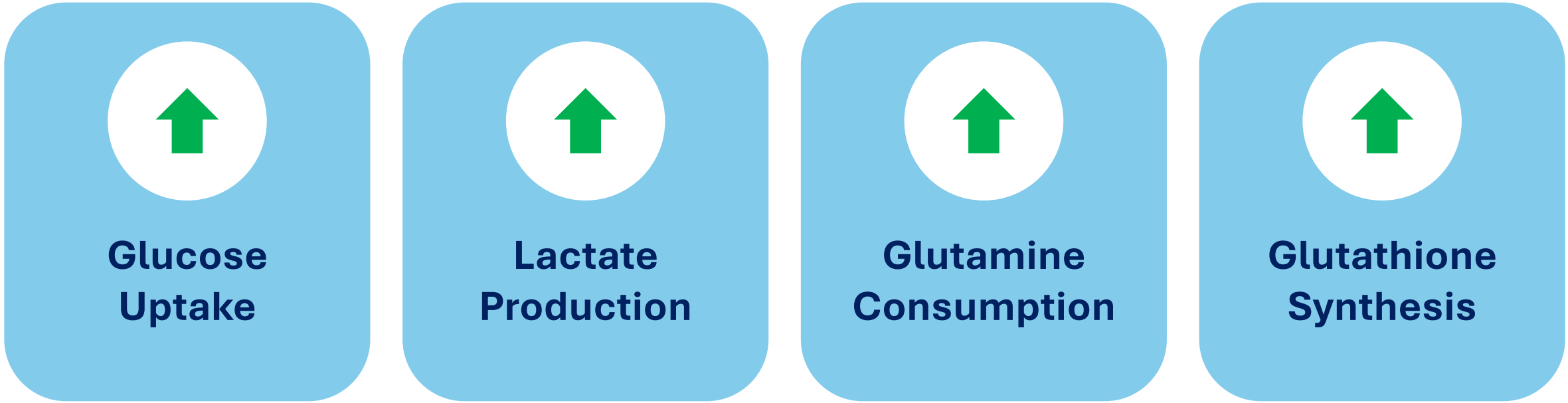


Suhail, S., Zajac, J., Fossum, C. et al. Role of Oxidative Stress on SARS-CoV (SARS) and SARS-CoV-2 (COVID-19) Infection: A Review. Protein J 39, 644–656 (2020). https://doi.org/10.1007/s10930-020-09935-8

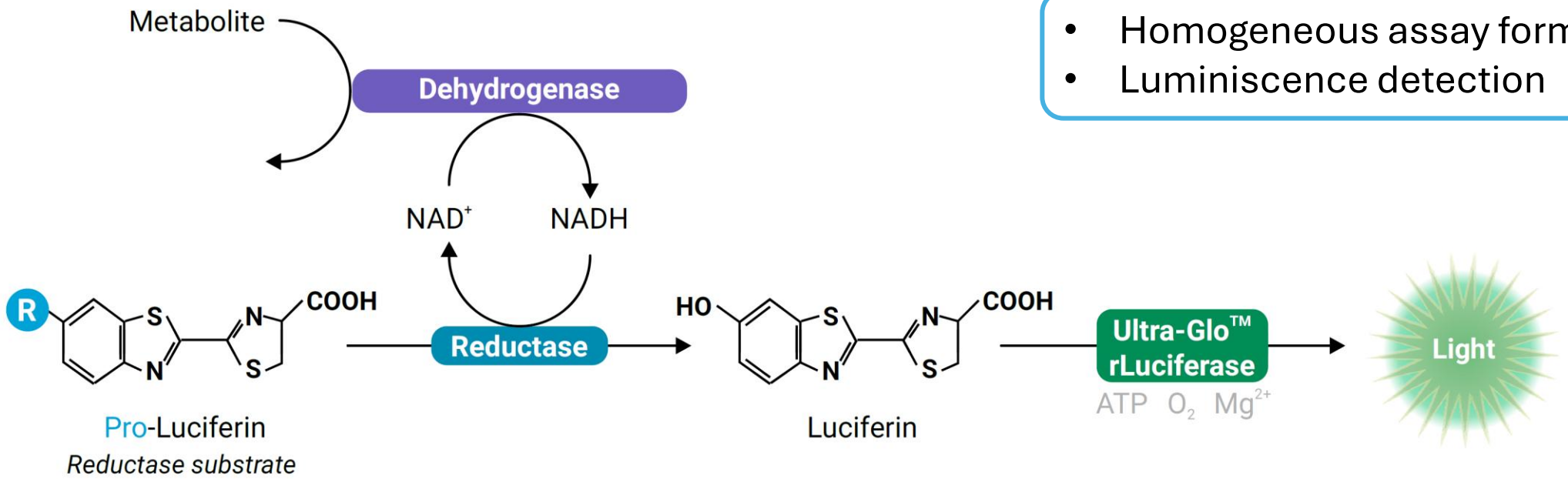
ROS = reactive oxygen species

Conclusion: 1st part

Specific metabolic changes for cancer cells



Assays for Assesing Metabolic Changes



- Homogeneous assay format
- Luminiscence detection

„One reaction does it all, one reaction commands it, one reaction answers all, and in the workflow binds it.“

Assays for Assesing Metabolic Changes

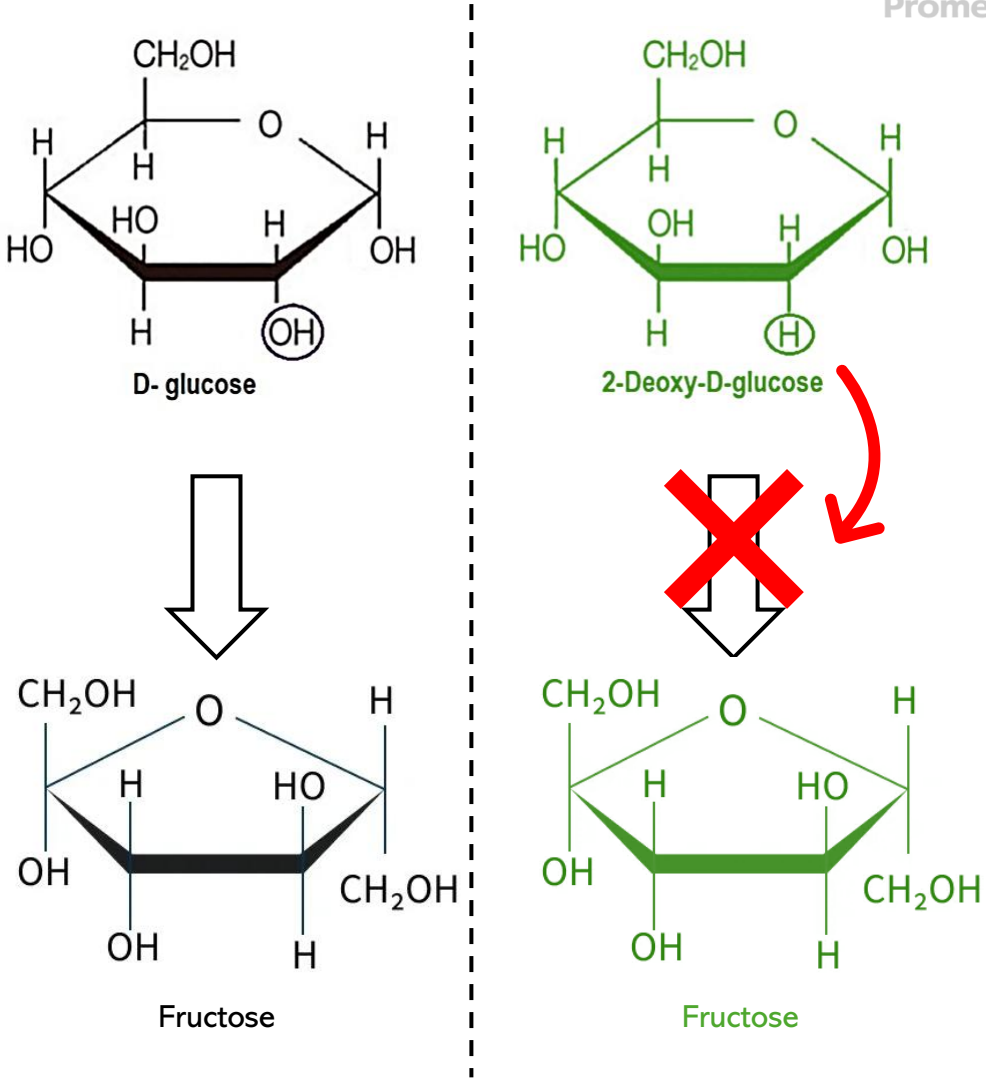
Glucose uptake	**Glucose Uptake-Glo™ Assay**	**Glycolysis monitoring**
Lactate production	**Lactate-Glo™ Assay**	
Glutamine consumption	Glutamate-Glo™ Assay; **Glutamine/Glutamate-Glo™ Assay**	
Redox balance	ROS-Glo™ H2O2 Assay; **GSH/GSSG-Glo™ Assay**; NAD/NADH-Glo™ & NADP/NADPH-Glo™	

Glucose Uptake-Glo™ Assay

- Measures uptake of 2-Deoxy-D-glucose (2-DG)

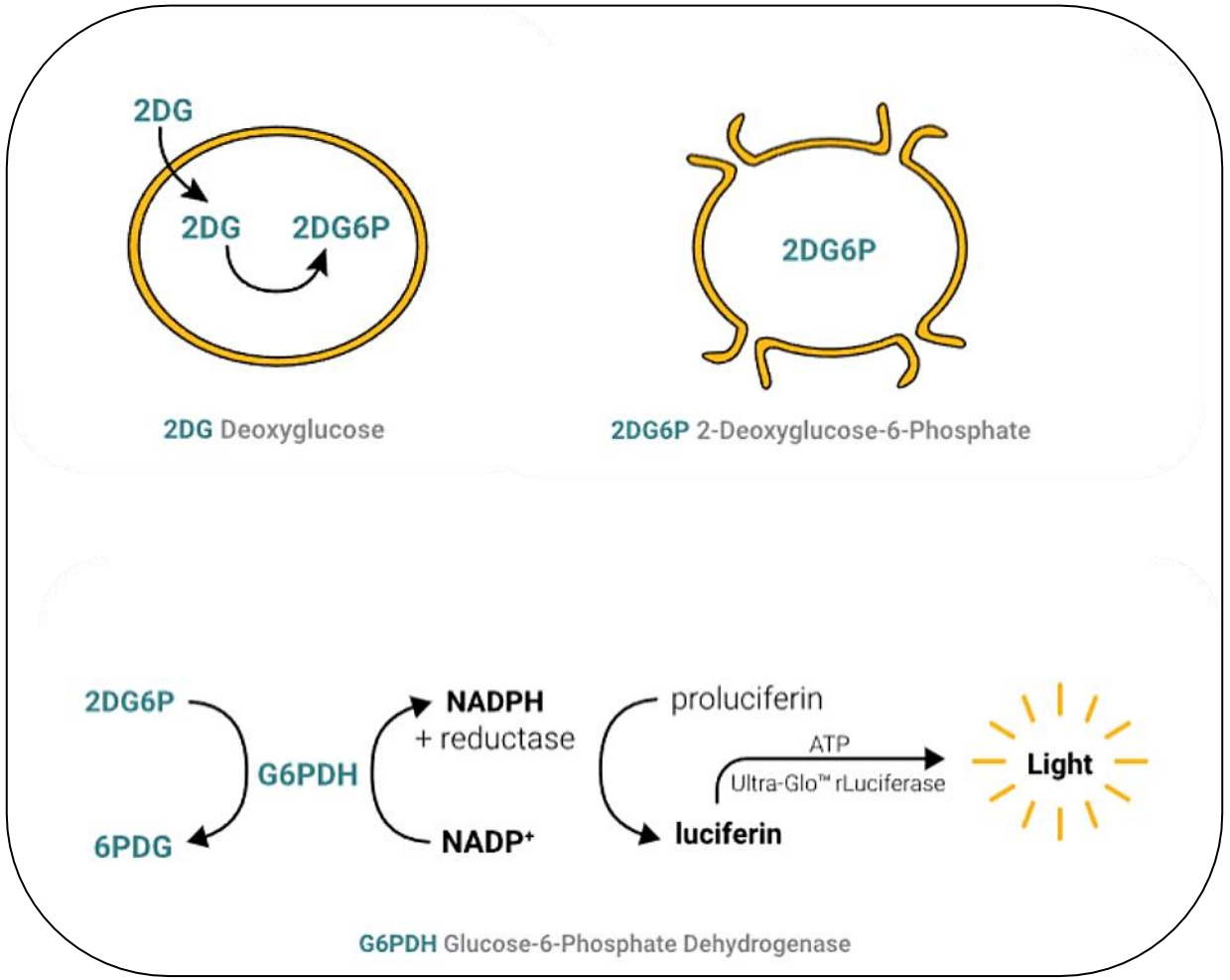
- 2-DG-6-P is **unable** to undergo isomerization to **fructose-6-P**

2-DG-6-P is **accumulated** in the cell

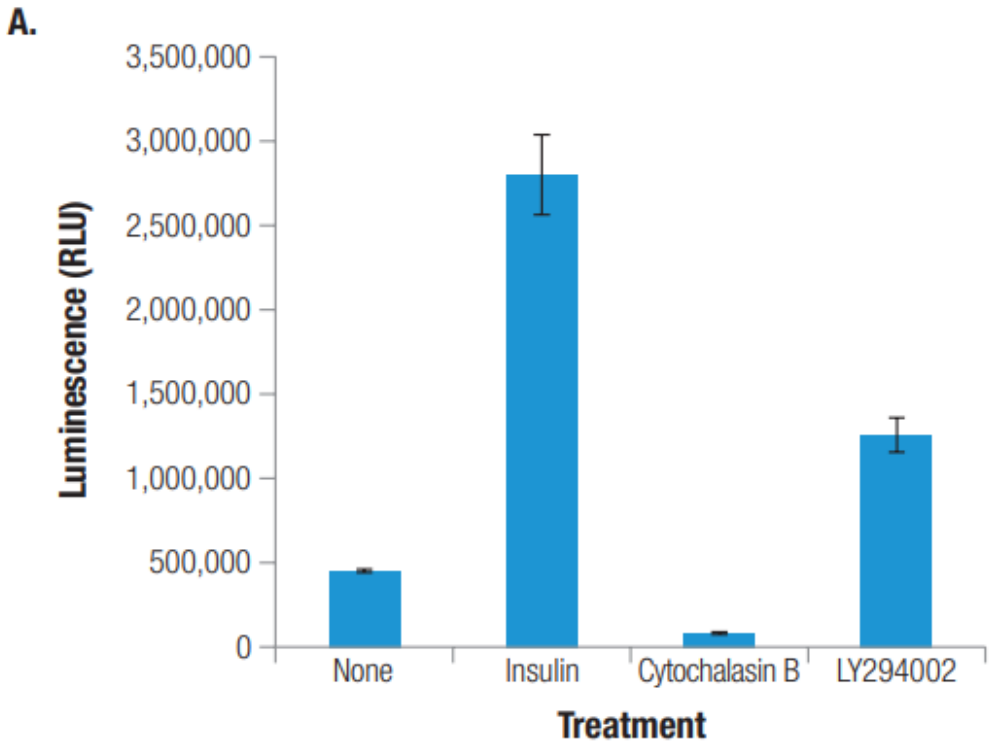


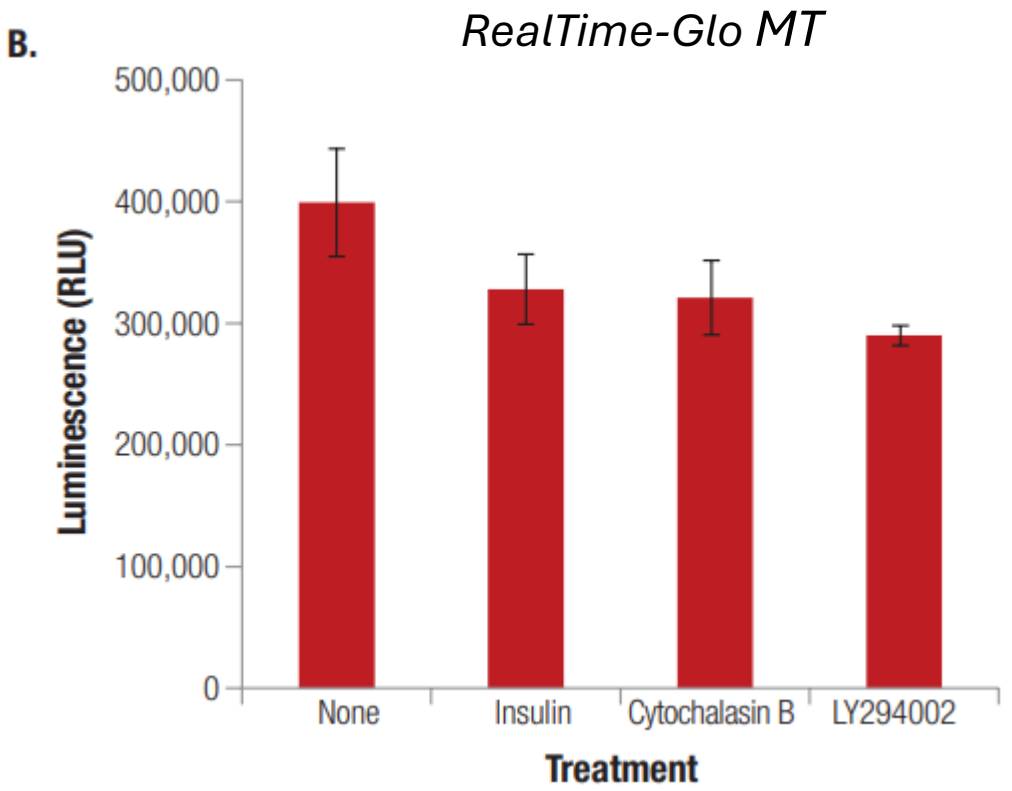
Pajak, B.; Siwiak, E.; Sołtyka, M.; Priebe, A.; Zieliński, R.; Fokt, I.; Ziemniak, M.; Jaśkiewicz, A.; Borowski, R.; Domoradzki, T.; et al. 2-Deoxy-d-Glucose and Its Analogs: From Diagnostic to Therapeutic Agents. Int. J. Mol. Sci. 2020, 21, 234. https://doi.org/10.3390/ijms21010234

Glucose Uptake-Glo™ Assay



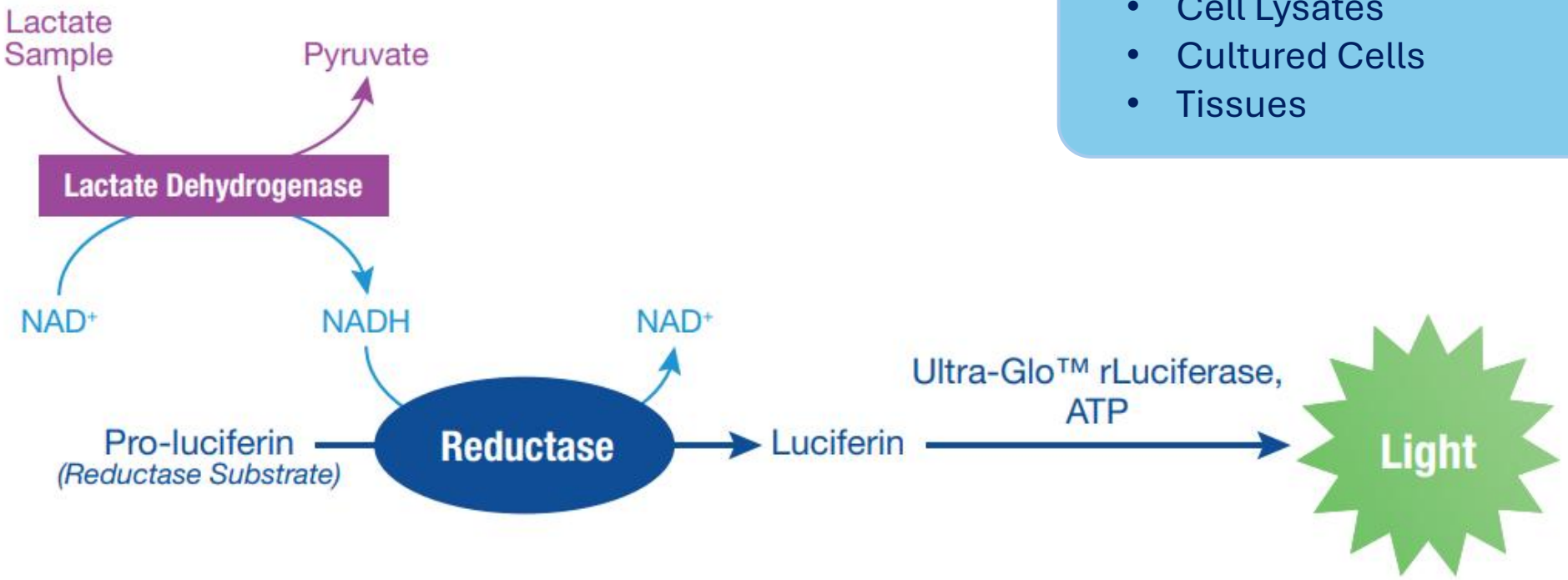
Glucose Uptake-Glo™ Assay





- Glucose uptake changes in **adipocytes** after treatment
- **Cytochalasin B** – glucose transporter inhibitor
- **LY294002** inhibits **PI3K** signalling → decreases glucose uptake

Lactate-Glo™ Assay

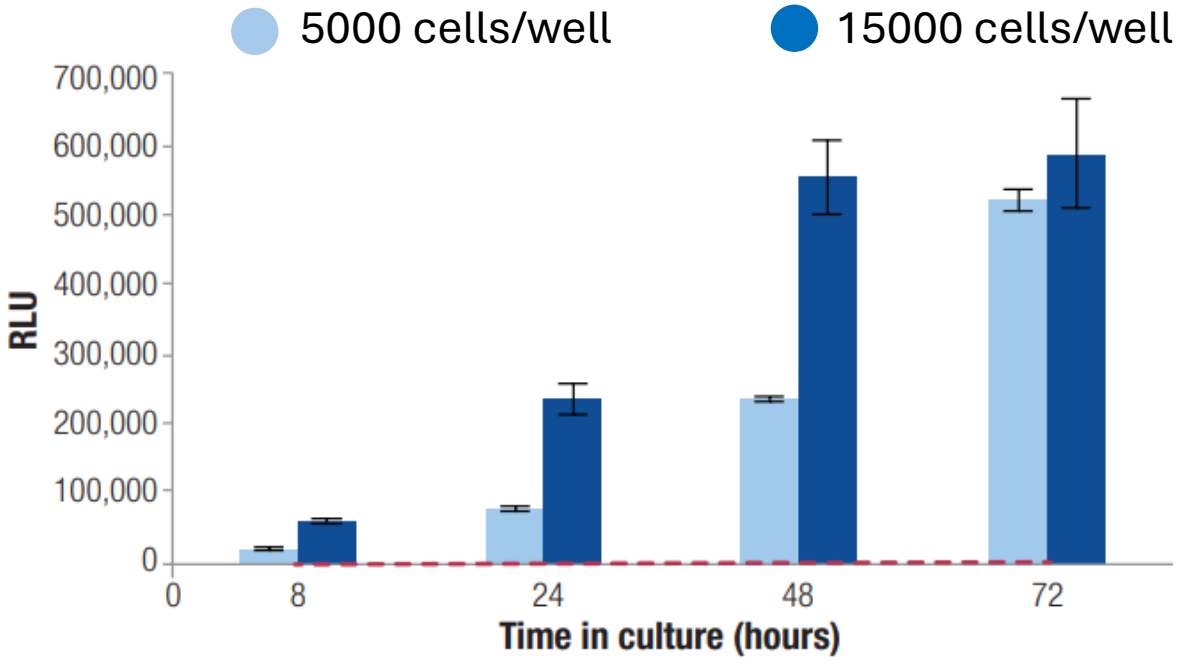


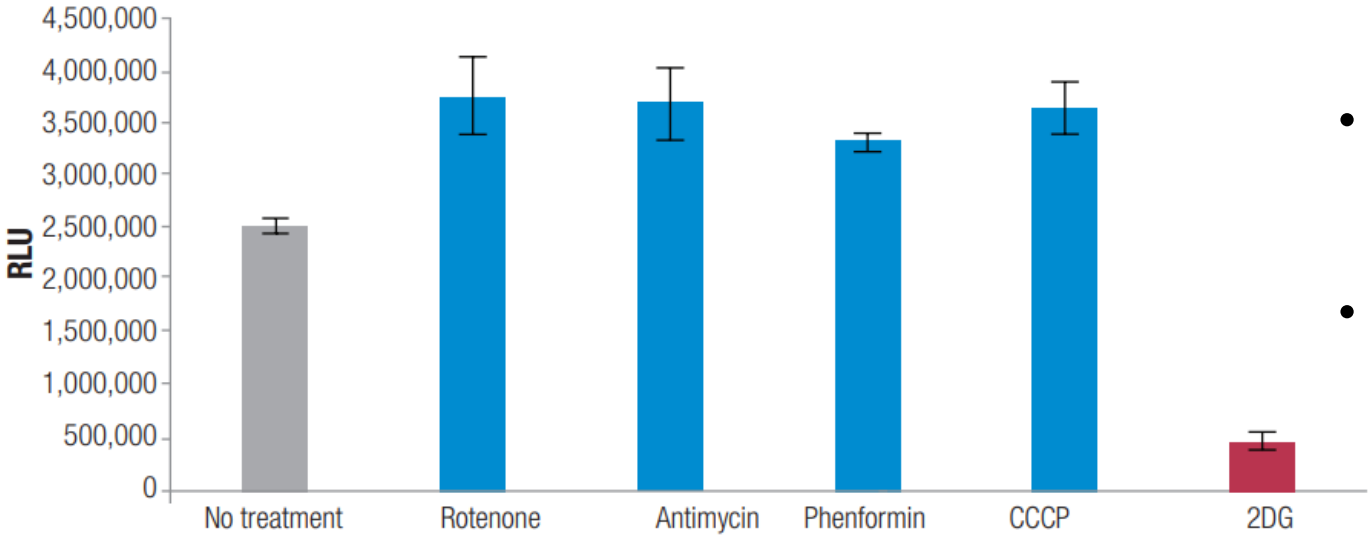
Sample

- Cell Culture Media
- Plasma, serum
- Cell Lysates
- Cultured Cells
- Tissues

Lactate-Glo™ Assay

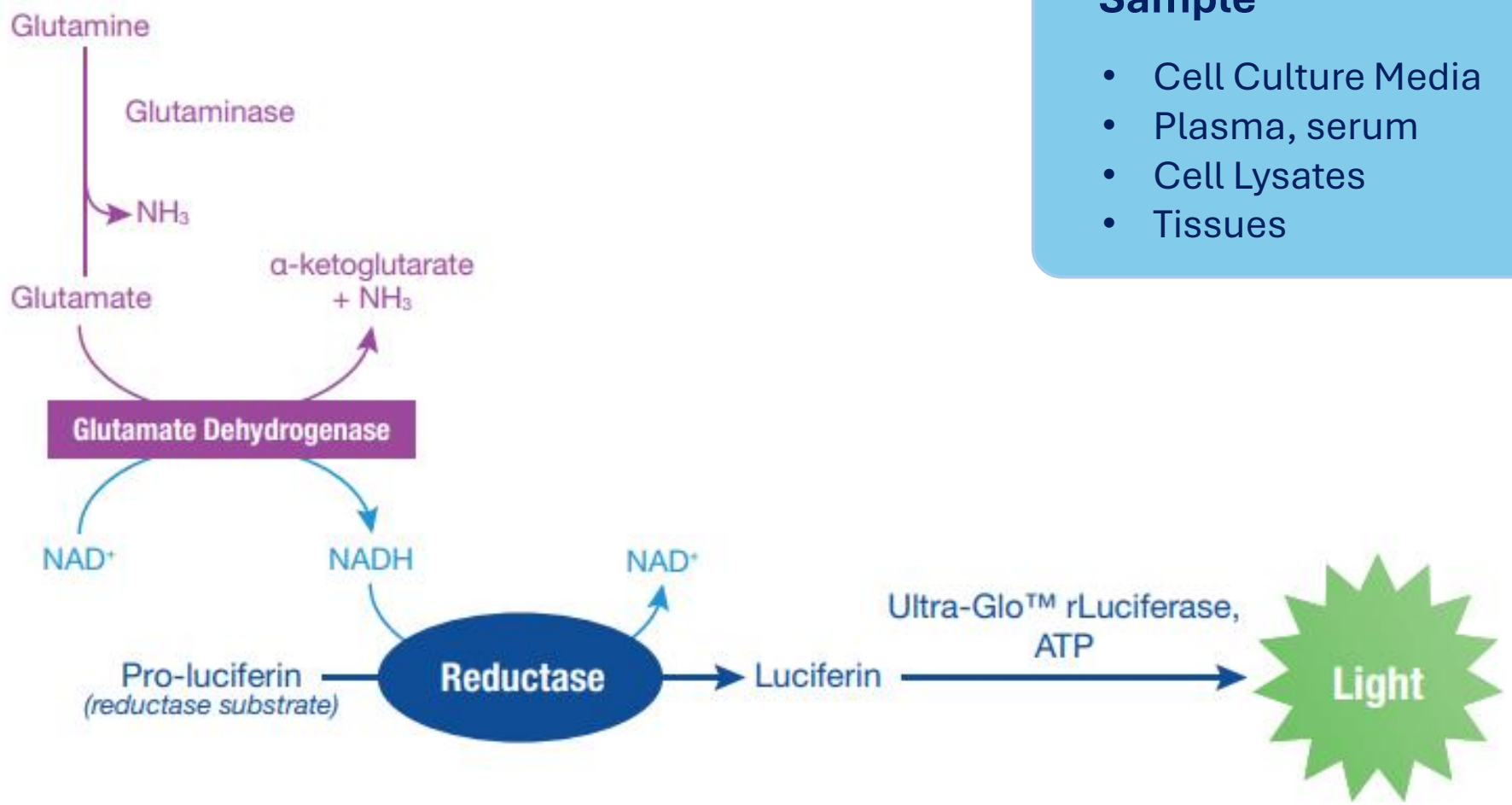
- Lactate secretion in **A549** cells
- DMEM with 5mM glucose





- **A549** cells treated with **mitochondrial/glycolysis inhibitors**
- **4mM** glucose added after treatment

Glutamine/Glutamate-Glo™ Assay

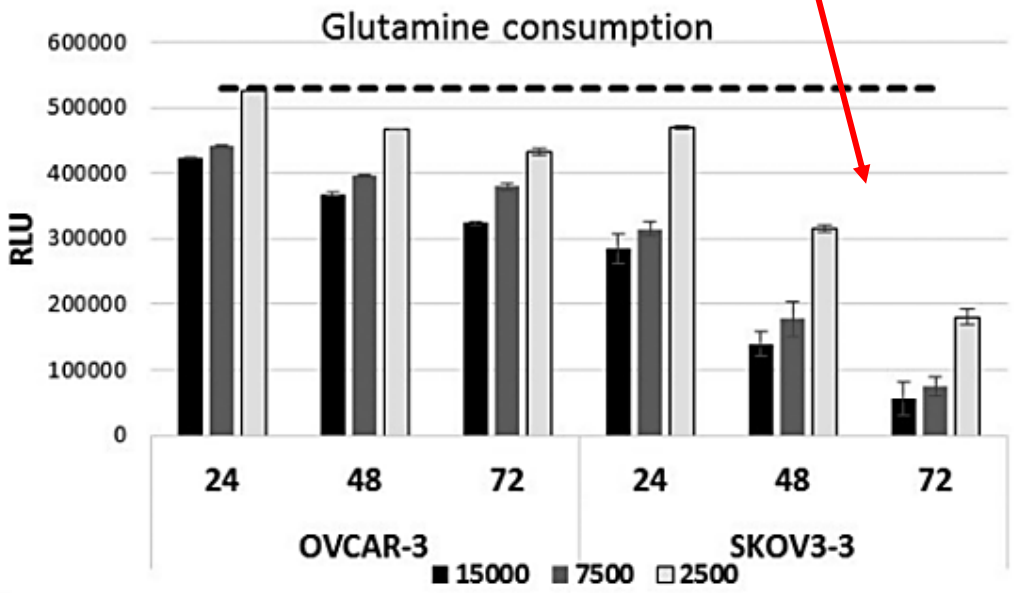


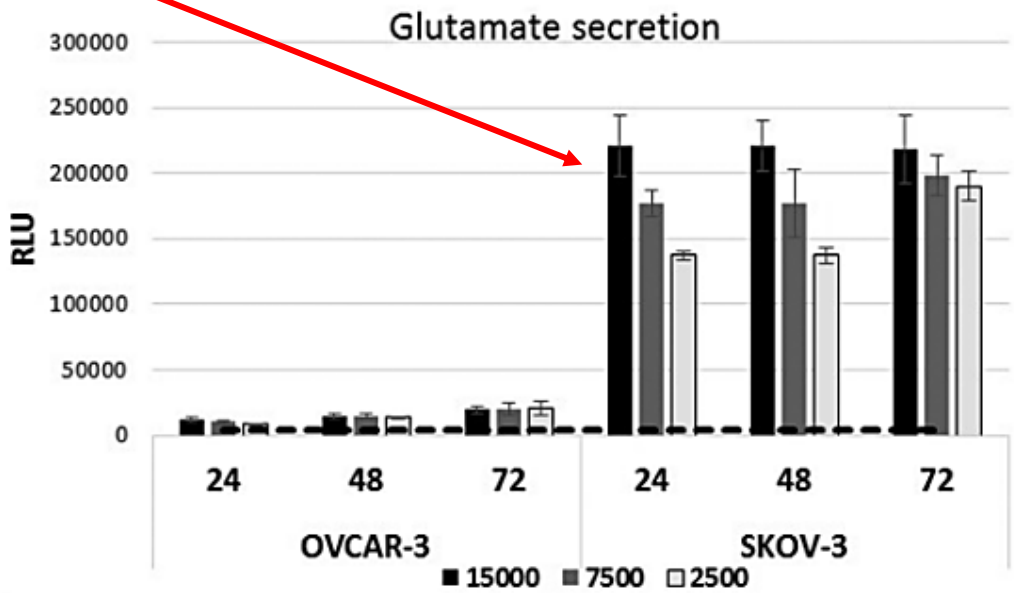
Sample

- Cell Culture Media
- Plasma, serum
- Cell Lysates
- Tissues

Glutamine/Glutamate-Glo™ Assay

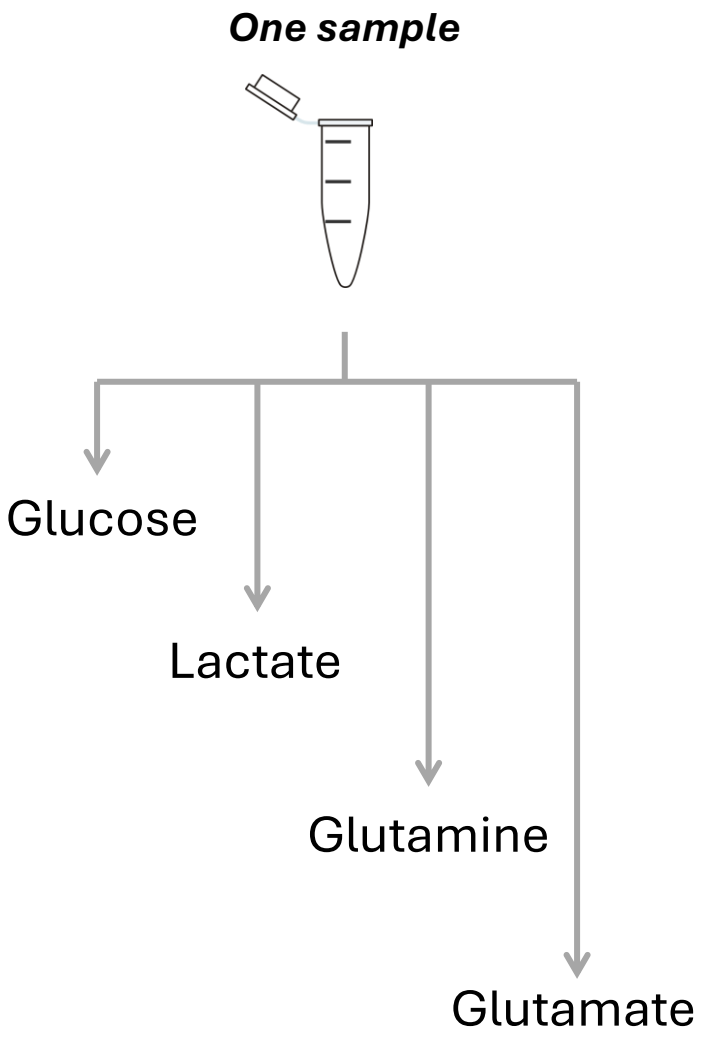
- Ovarian cancer cell lines OVCAR-3 & SKOV-3
- **SKOV-3** → highly glutamin **dependent**

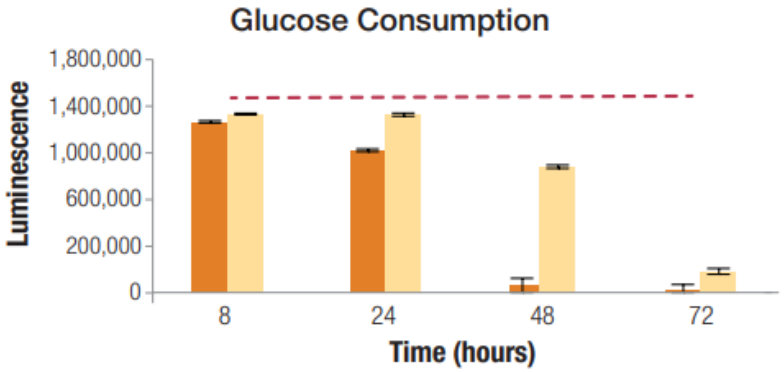


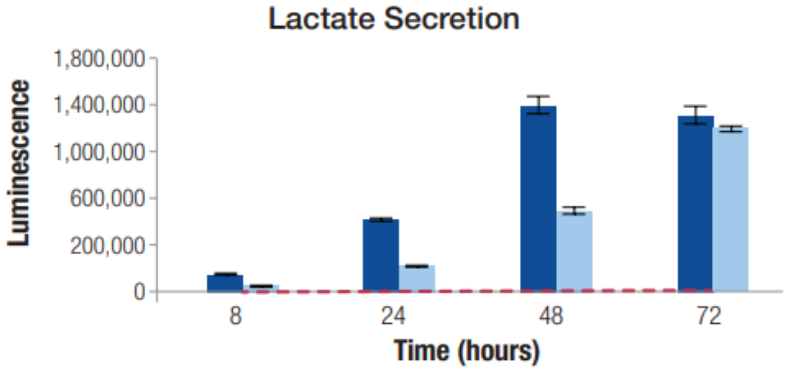


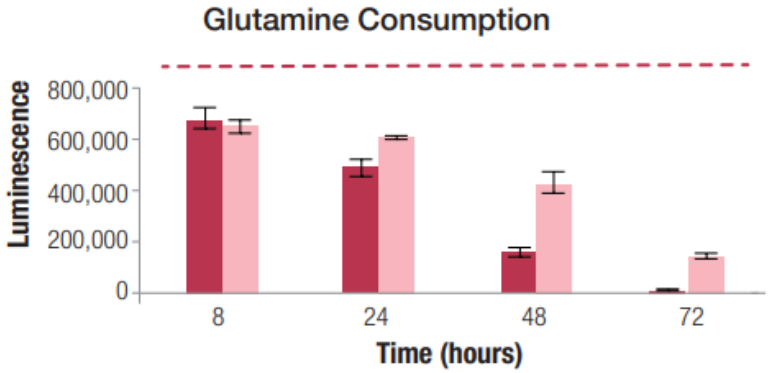
Leippe D, Sobol M, Vidugiris G ...Bioluminescent Assays for Glucose and Glutamine Metabolism: High-Throughput Screening for Changes in Extracellular and Intracellular Metabolites
SLAS Discovery, 22, 366-377

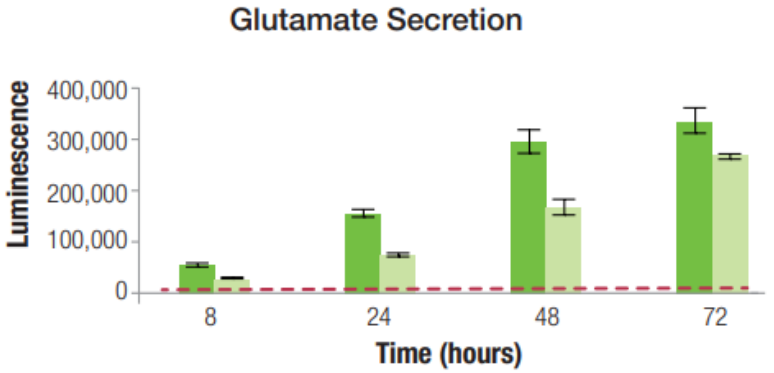
One Sample, Multiple Metabolites





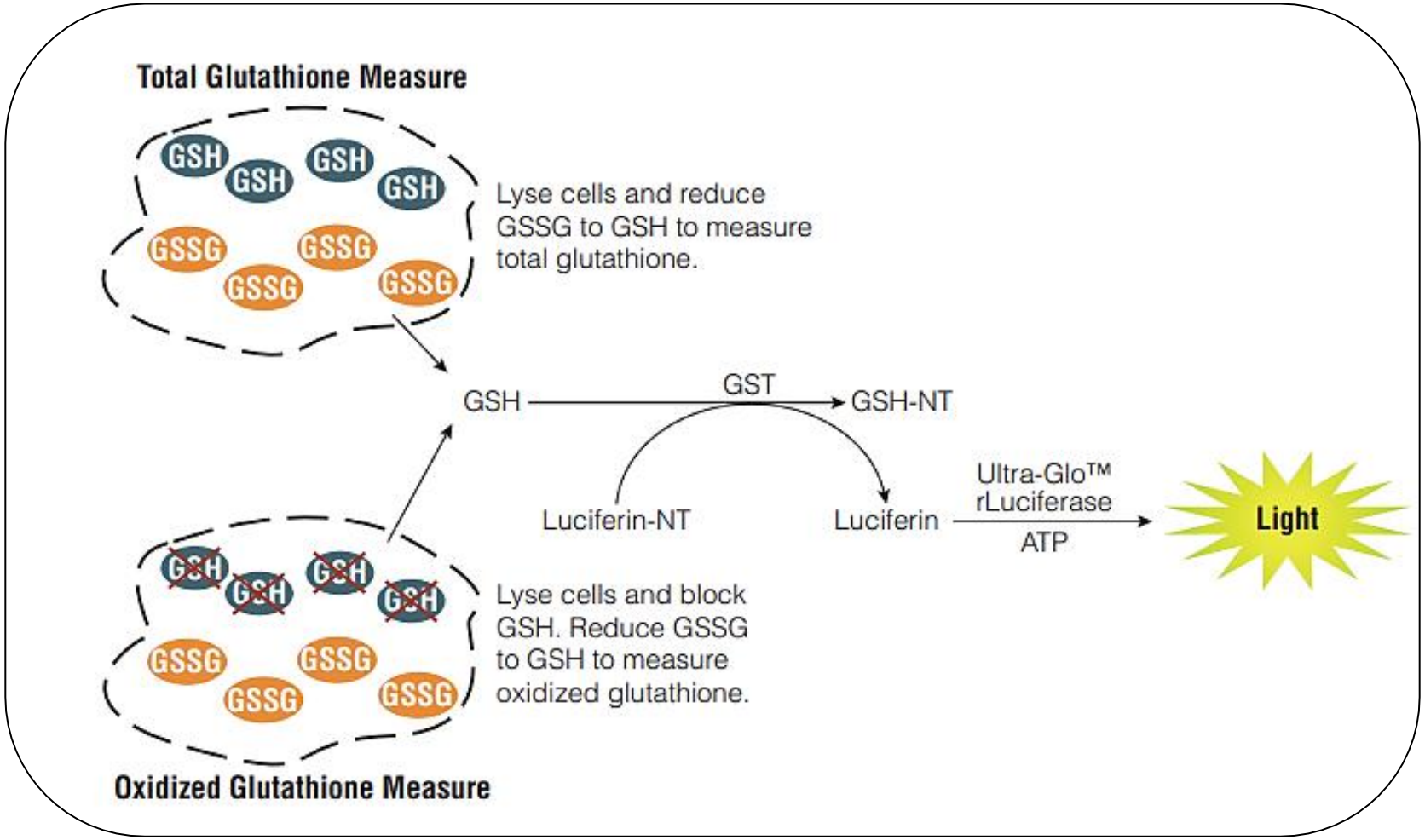






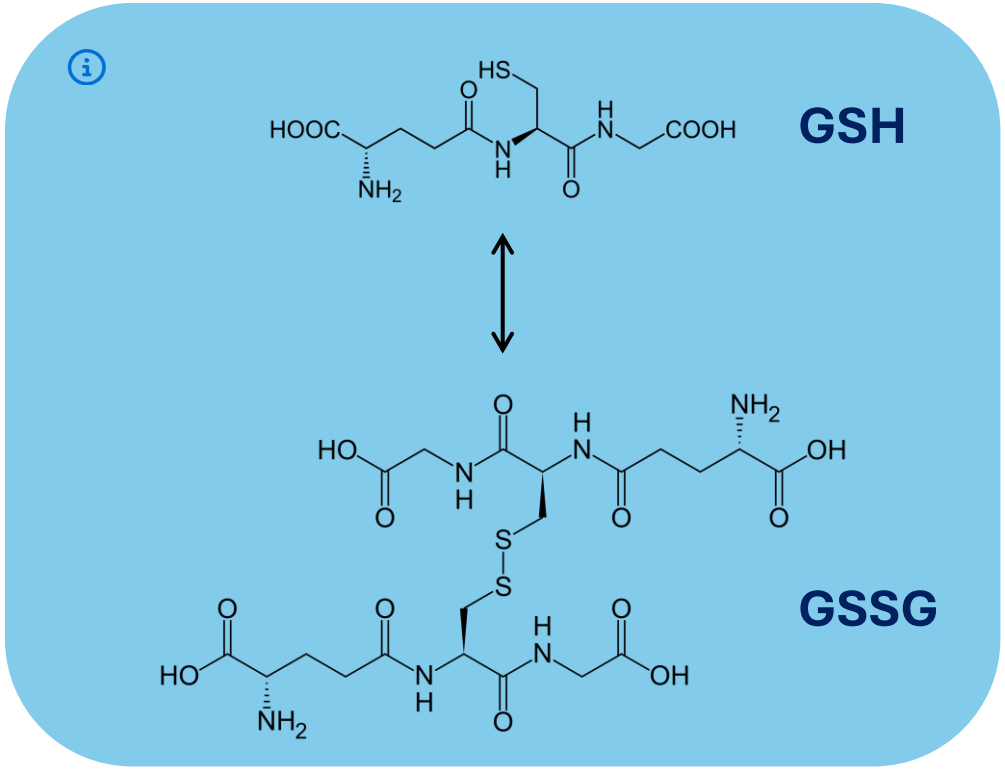
Medium from **A549** cells was collected, diluted and frozen at –20°C. After thawing, aliquots were transfered to 384-WP and each metabolite was detected using either the **Lactate-Glo™, Glucose-Glo™, or Glutamine/Glutamate-Glo™ Assays**, respectively.

GSH/GSSG-Glo™ Assay



GSH/GSSG-Glo™ Assay

- Cells treated with 40uM **menadione**
- Menadione → induces the formation of ROS
- Level of GSH and thus GSH/GSSG ratio **decreases**



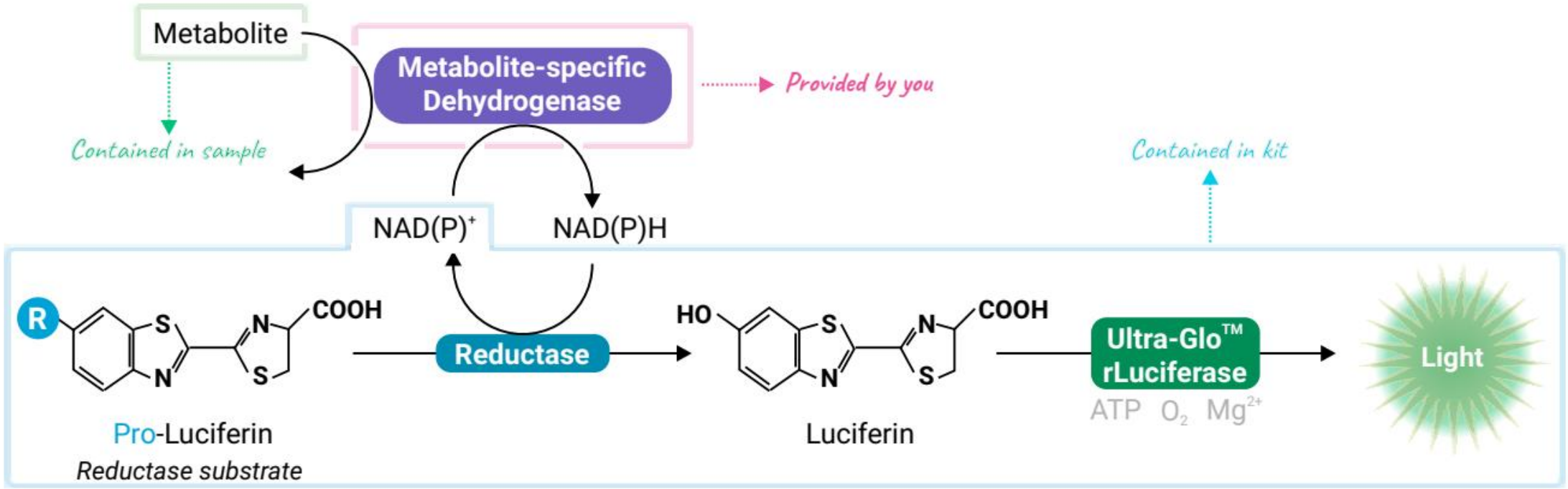
		GSH/GSSG Ratio	
Cell Type	**Cells/Well**	**Vehicle Control Cells**	**Menadione-Treated Cells**
HeLa	5,000	65.8	4.2
Rat Hepatocytes	20,000	21.8	3.1
HepG2	5,000	75.2	3.5
Jurkat	10,000	15.0	3.8

Assays for Assesing Metabolic Changes

Glucose uptake	**Glucose Uptake-Glo™ Assay**	**Glycolysis monitoring**
Lactate production	**Lactate-Glo™ Assay**	
Glutamine consumption	Glutamate-Glo™ Assay; **Glutamine/Glutamate-Glo™ Assay**	
Redox balance	ROS-Glo™ H2O2 Assay; **GSH/GSSG-Glo™ Assay**; NAD/NADH-Glo™ & NADP/NADPH-Glo™	

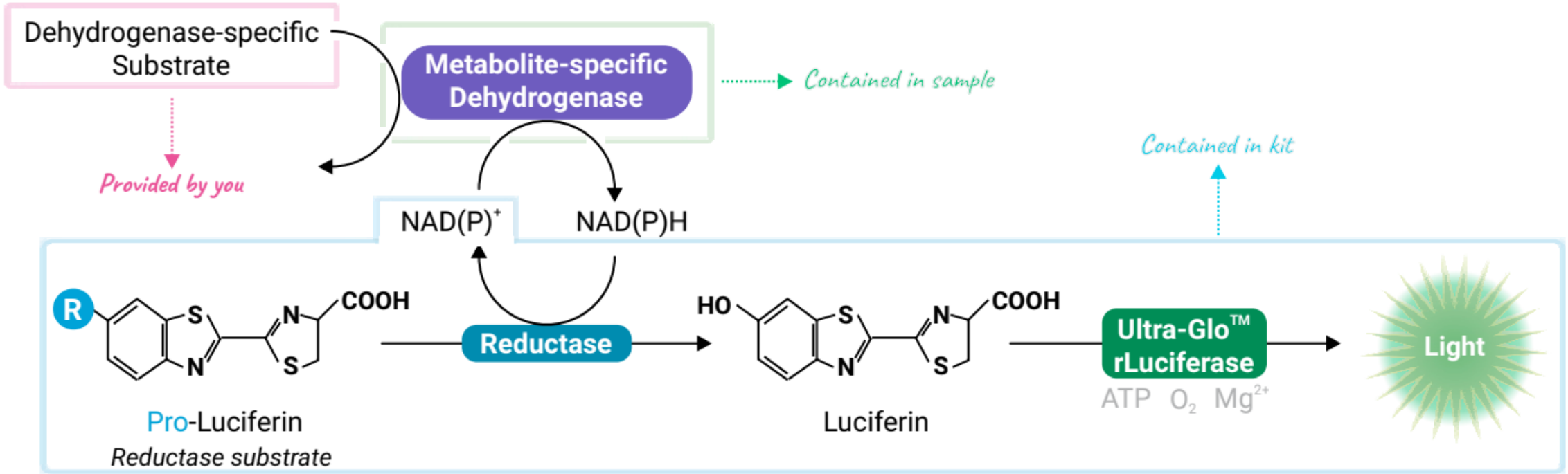
Build Your Own Metabolism Assay

Metabolite-Glo



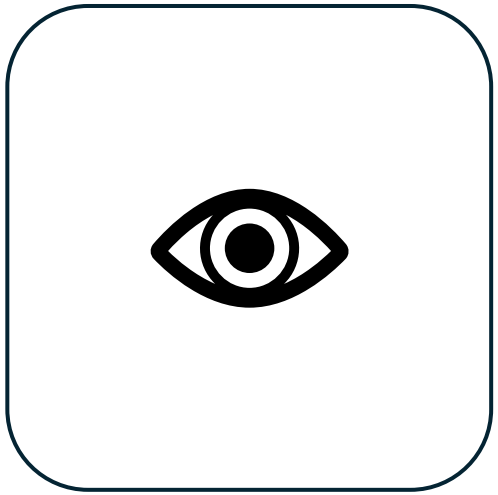
Build Your Own Metabolism Assay

Dehydrogenase-Glo

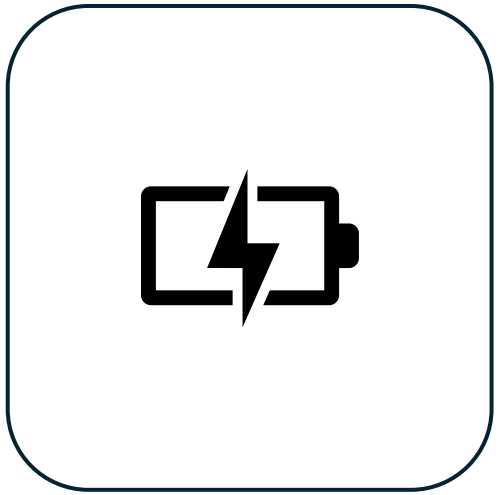


Label-free Cell Analysis

Imaging

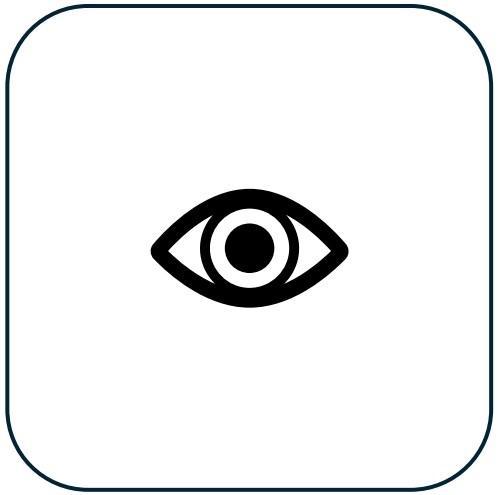


Current

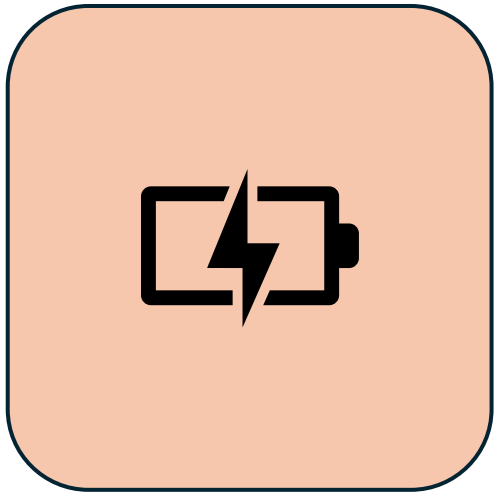


Label-free Cell Analysis

Imaging



Current

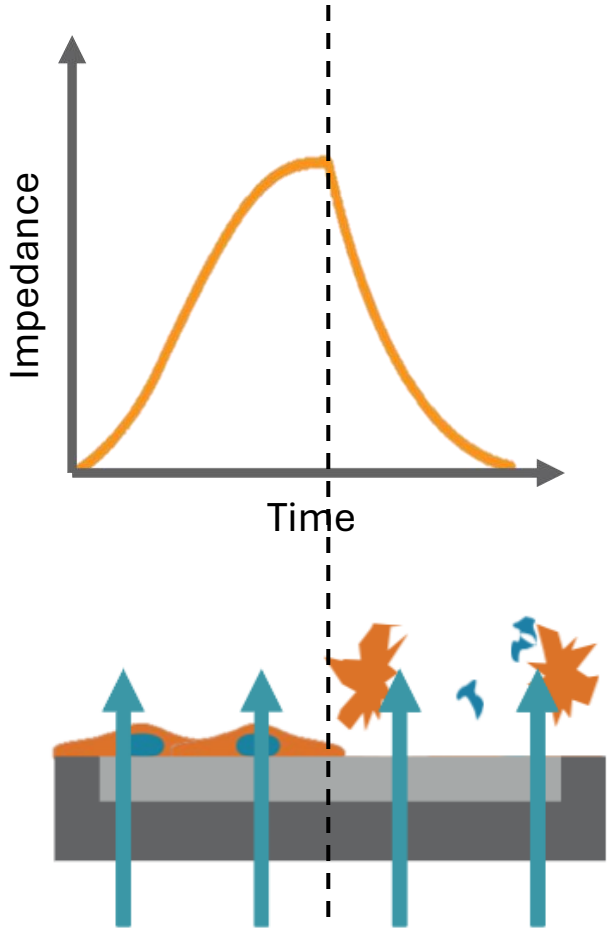


Impedance: resistance measurement

- Measures how easily signal passes the electrode-cell interface = **resistence**

Impedance ↑

- *Coverage increases*
- *Attachment increases*



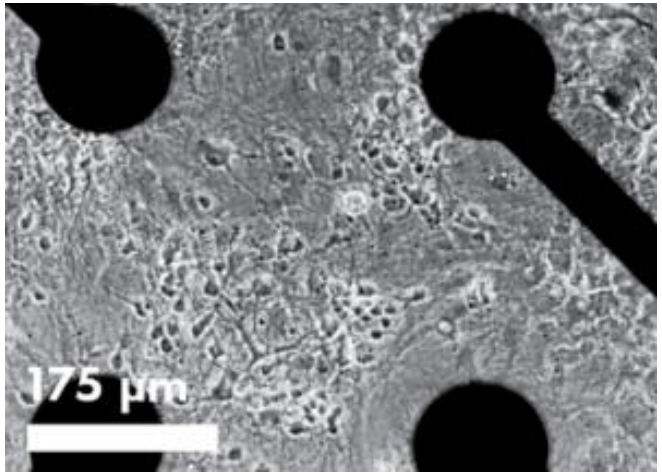
Impedance ↓

- *Dettachment*
- *Change in morphology*
- *Monolayer breaks down*

MEA: Microelectrode Array

- Measures electrical activity
- Explores activity of **neurons, cardiomyocytes, muscle & retinal cells**
- MEA plates with microelectrodes

CytoView MEA plates



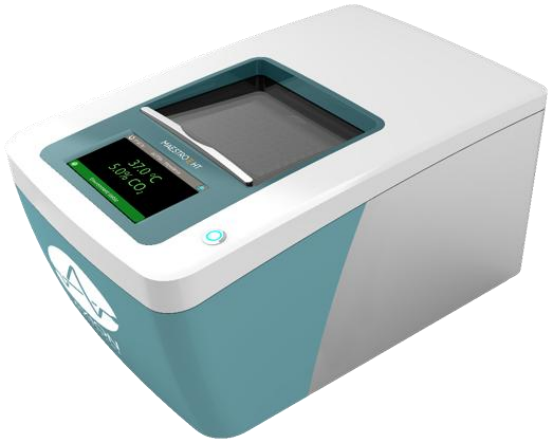
Applications

- Spontaneous activity
- Drug-induced changes
- Maturation
- Network dynamic

https://www.axionbiosystems.com/products/mea/cytoview-mea-plate

Maestro Family

Maestro Z



- ***Impedance-based*** *viability measurement*
- *Assess cells proliferation, viability, cytotoxicity*
- Built-in **incubation chamber**

Maestro MEA

- ***MEA-based*** *monitoring of neurons, cardiomyocytes, muscle & retinal cells*
- Built-in **incubation chamber**
- *Maestro Volt, Edge & Pro*

Maestro Family

Maestro Z

Maestro ZHT

Features	*Maestro Z*	*Maestro ZHT*
Throughput:	96-well	384- and 96-well
Environmental Controls:	Built-in	Built-in
GxP Compatible:	✓	✓
Barcode Plate Tracking:	✓	✓
Automation API:	✓	✓
Dimensions *(WxDxH)*:	280 x 413 x 225 mm	280 x 452 x 225 mm

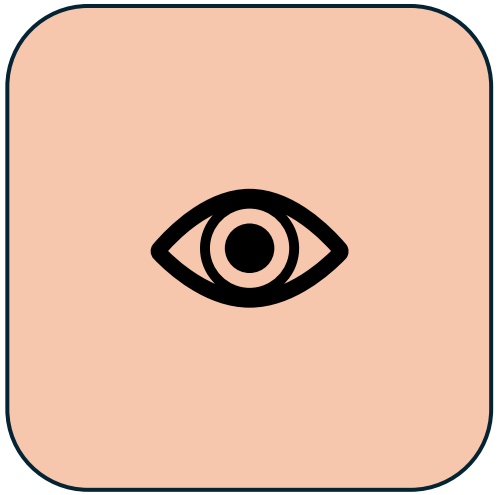
Maestro Pro Maestro Edge Maestro Volt

Features	*Maestro Pro*	*Maestro Edge*	*Maestro Volt*
Throughput (well format)	6, 24, 48, 96, 384*	6, 24, 96*	6
MEA Mode	✓	✓	✓
MEA Viability	✓	✓	
Impedance Mode	✓	✓	
Environmental Control	✓	✓	✓
Automation API	✓	✓	
Stimulation	Electrical & Optical	Electrical & Optical	Electrical
Omni Compatible	✓	✓	✓

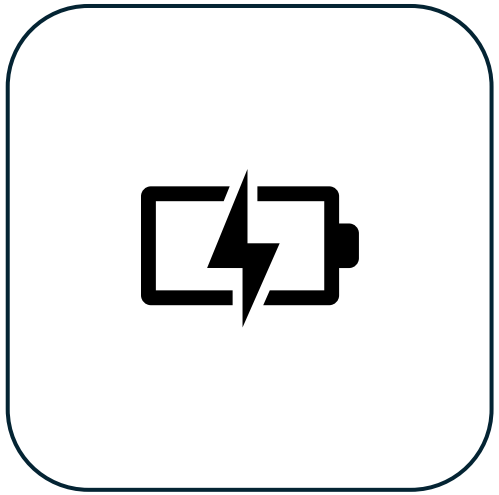
**Well format available in impedance only*

Label-free Cell Analysis

Imaging



Current



Lux3: Microscop for Your Incubator

- Compact live-cell imaging microscope
- Scans fixed area
- Compatible with all vessel types – plates, flasks, Petri dish etc.
- Brightfield + optional fluorescence with red and green channels

Applications

- *Confluency*
- *Scratch assays*
- *Fluorescence assays*

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