

# Cell-based Assays for Energy Metabolism and Oxidative stress

Jaroslav Icha, PhD

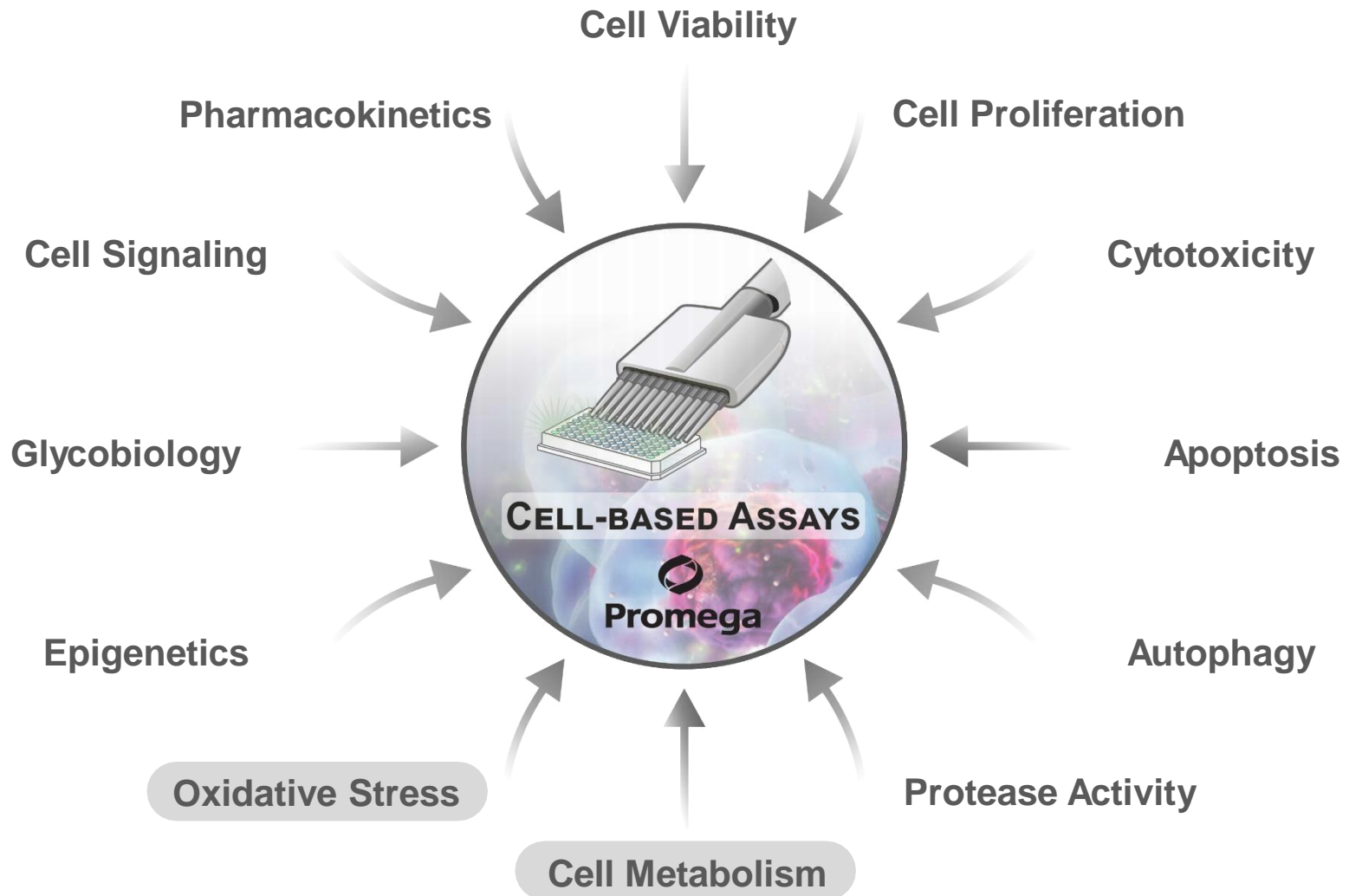
Application Specialist

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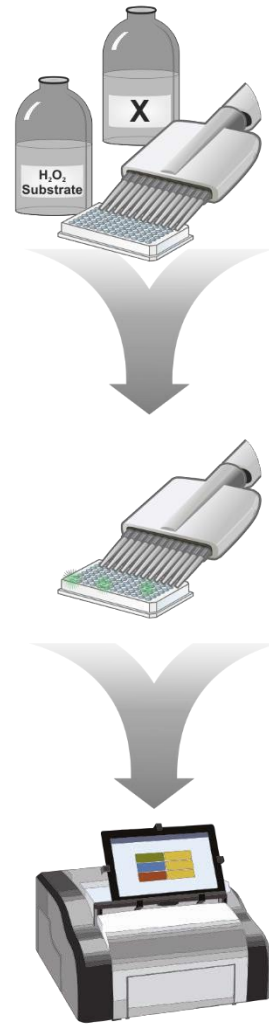
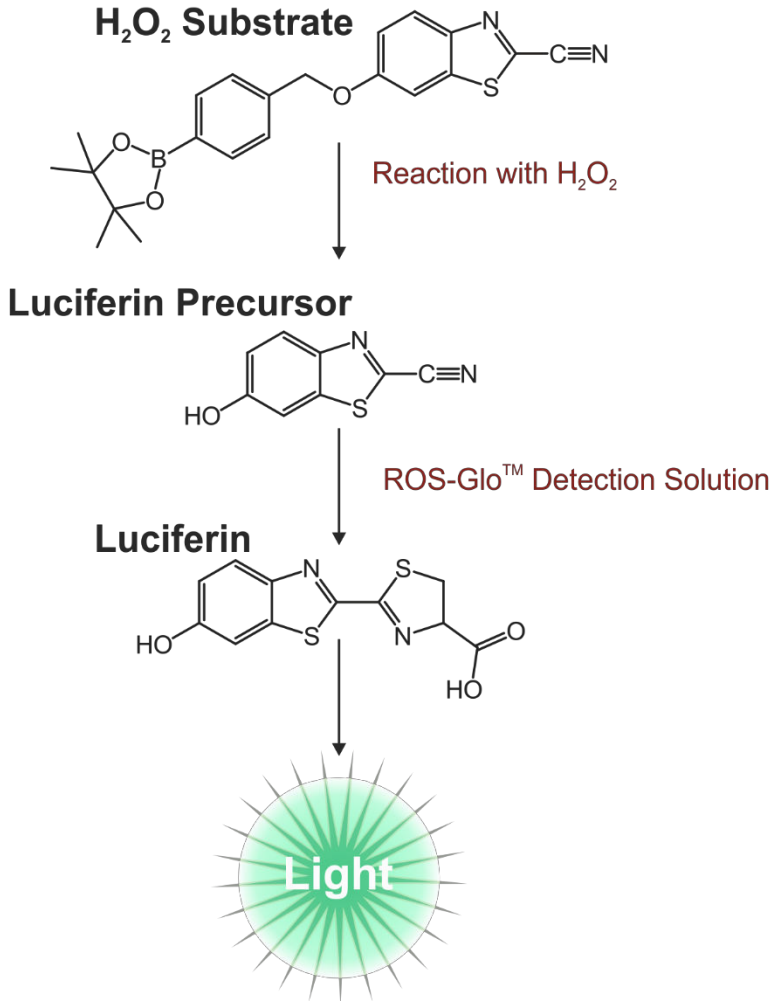


# Promega's Cell-based Assay Portfolio





# ROS-Glo™ H<sub>2</sub>O<sub>2</sub> Assay Principle & Workflow



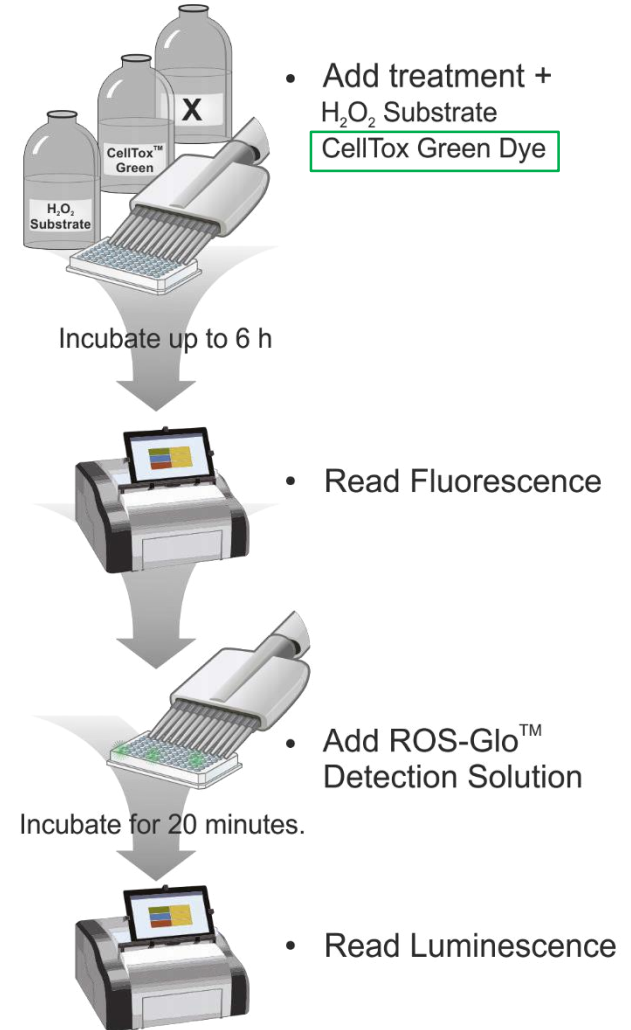
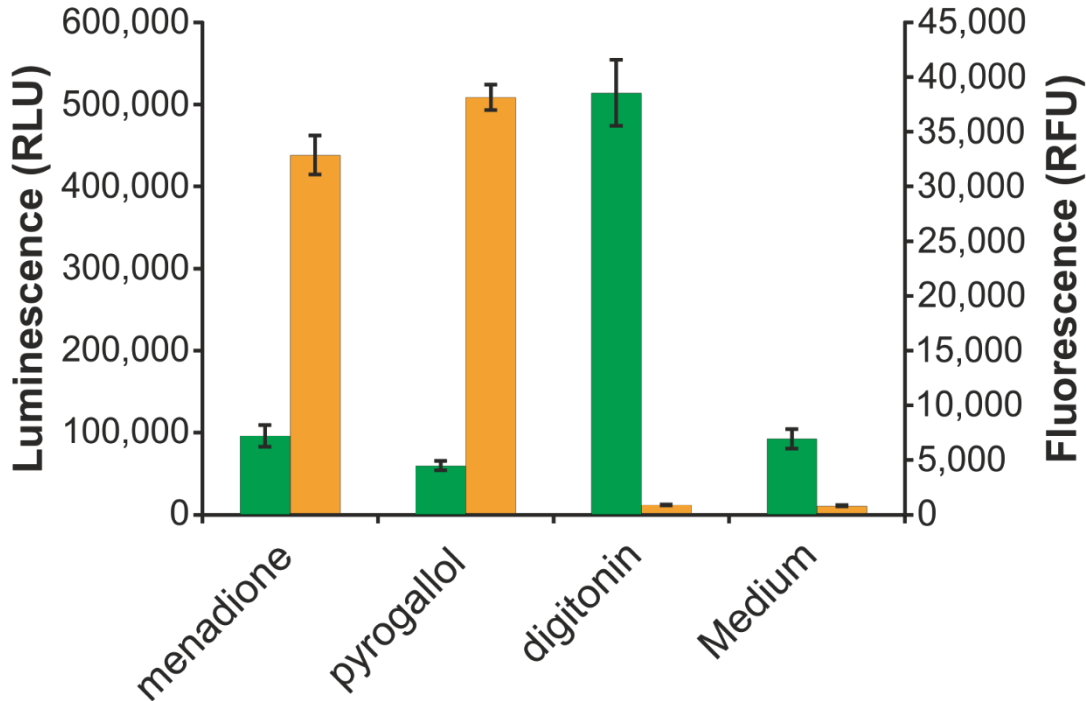
- Treat samples.
- Add H<sub>2</sub>O<sub>2</sub> Substrate Solution.
- Incubate for up to 6 hours.
- Add ROS-Glo™ Detection Solution to cells (lytic) or supernatant (non-lytic)
- Incubate for 20 minutes.
- Read Luminescence



# ROS-Glo™ and CellTox™ Green Multiplexing

HepG2 cells

- CellTox™ Green Cytotoxicity Assay
- ROS-Glo™ H<sub>2</sub>O<sub>2</sub> Assay





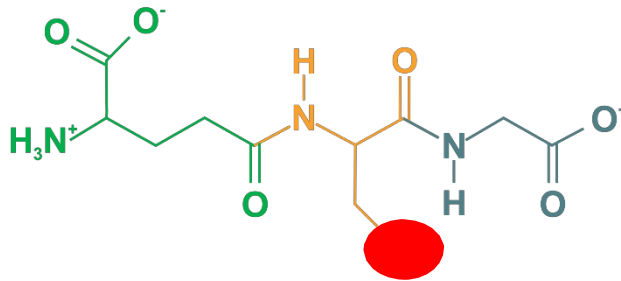
# GSH/GSSG-Glo™ Assay to Study Oxidative Stress





# GSH/GSSG-Glo™ Assay to Study Oxidative Stress

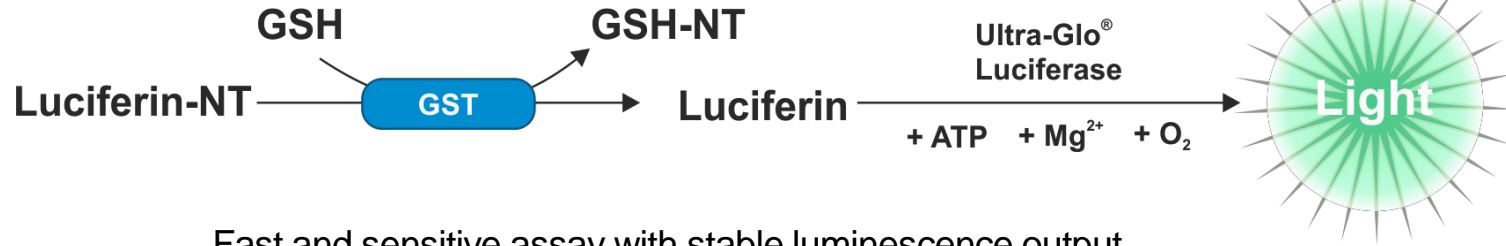
- Glutathione is a tripeptide (Glu-Cys-Gly)
- Synthesized in the cytosol by all mammalian cells
- Present at high concentrations (mM) in cells



- Reduced glutathione (GSH) serves as a cellular antioxidant
- Oxidized glutathione (GSSG) constitutes normally 2 – 10% of total glutathione
- GSH/GSSG ratio (↓) serves as a universal indicator for oxidative stress



# GSH/GSSG-Glo™ Assay Principle



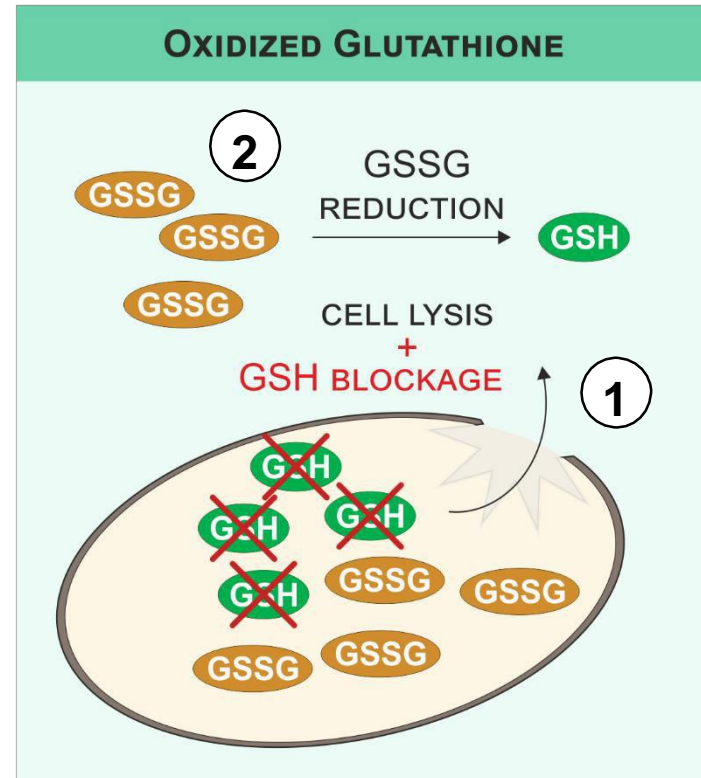
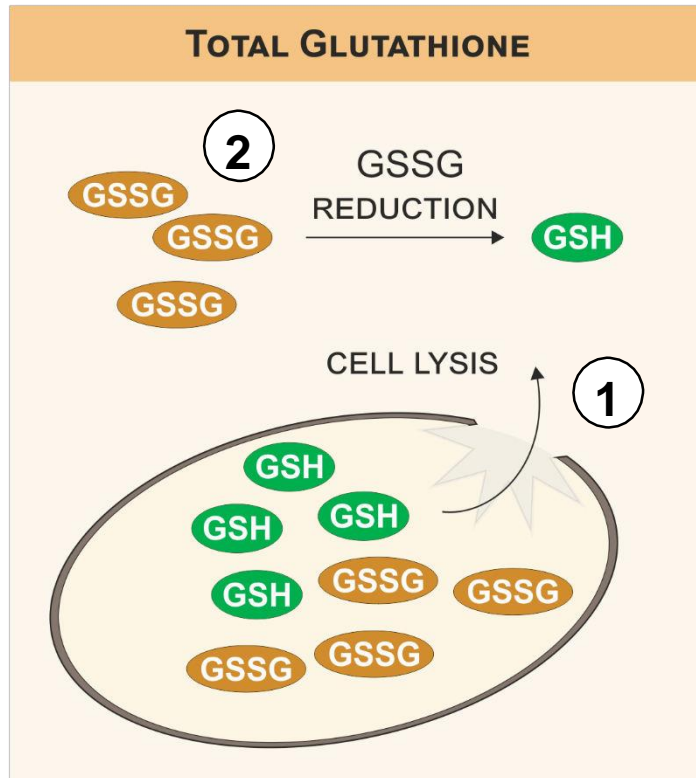
Fast and sensitive assay with stable luminescence output



# GSH/GSSG-Glo™ Assay Principle



$$GSH = total\ GSH - GSSG$$

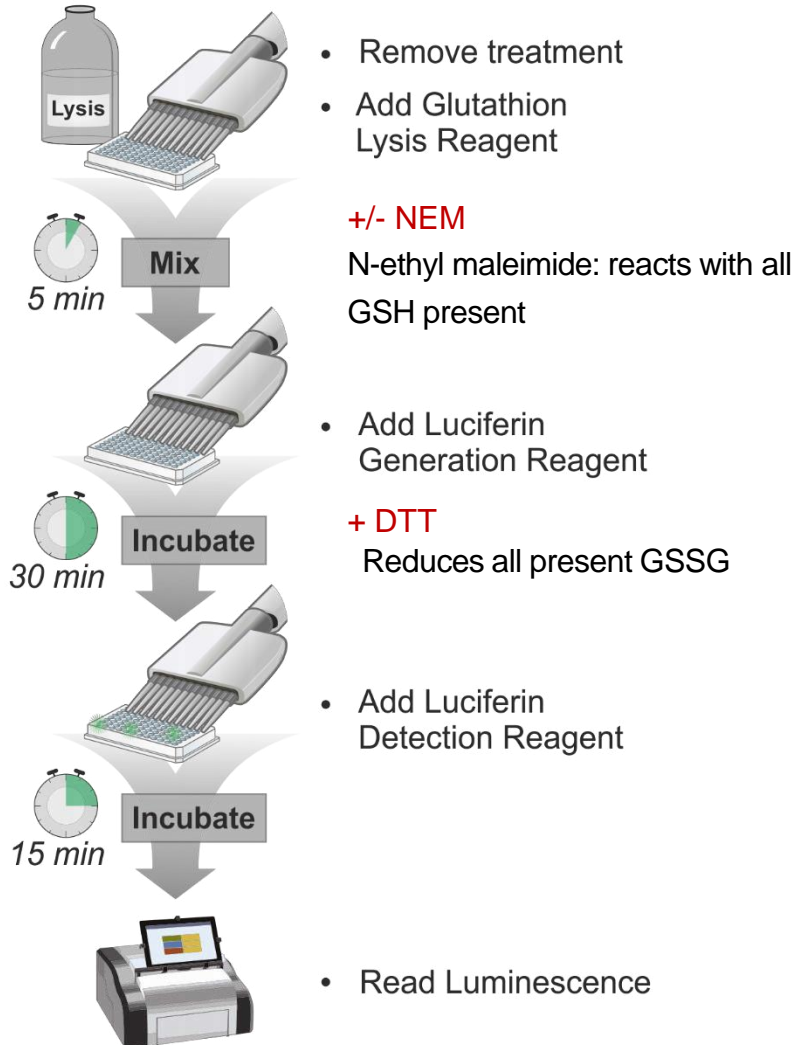






# GSH/GSSG-Glo™ Assay – Experimental pipeline

Medium needs to be completely removed.

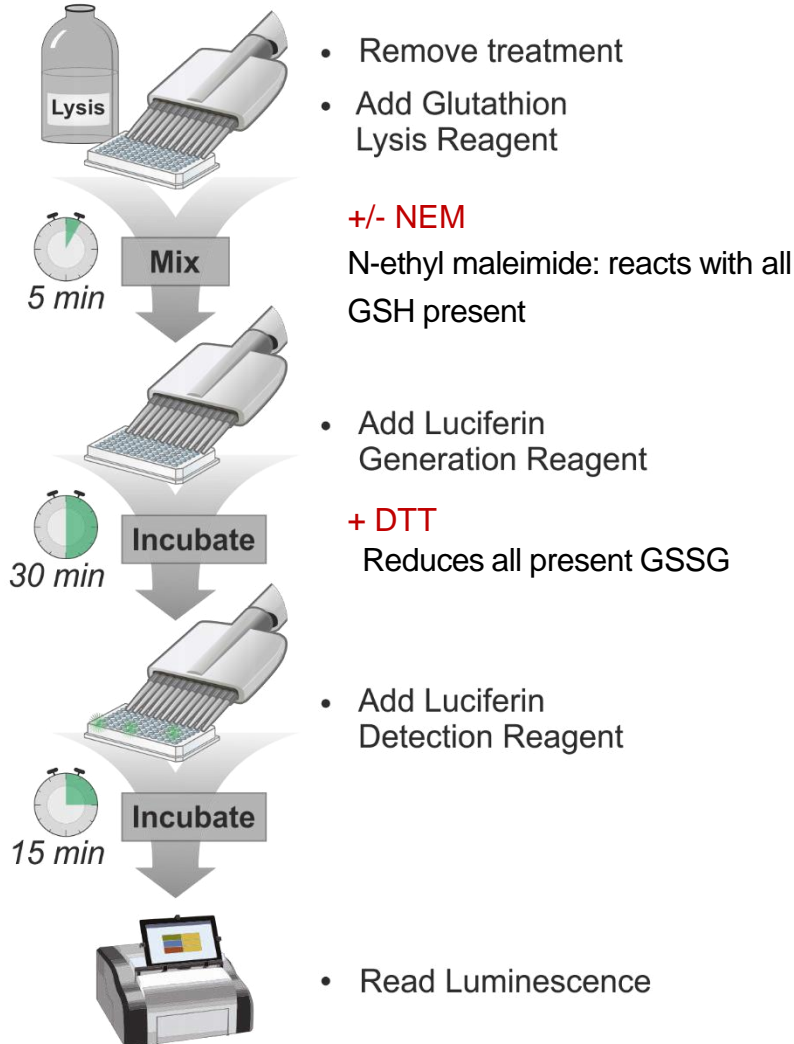


- For controls, the same recommendation as for the Caspase-Glo stays.
- It is good to prepare the calibration curve not only for absolute quantification, but also to check whether you are in the linear range of the assay.



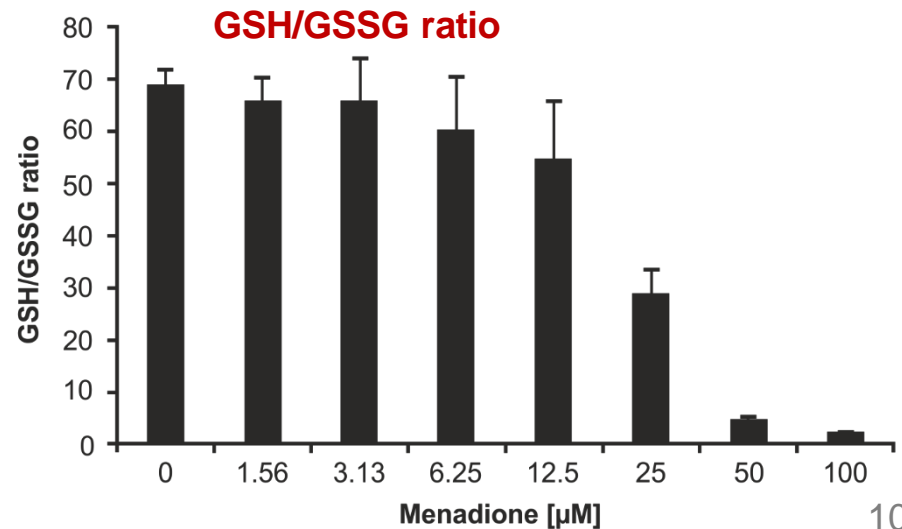
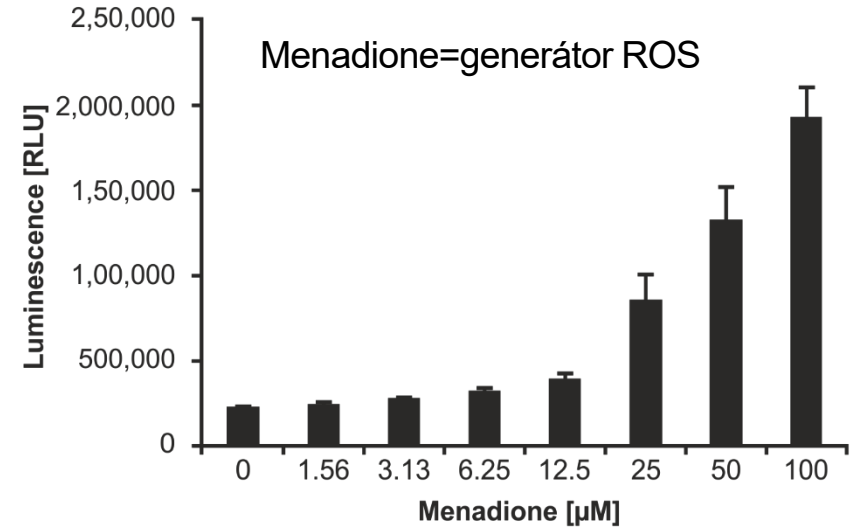
# GSH/GSSG-Glo™ Assay – Representative Data

Medium needs to be completely removed.



**GSSG**

**A549 cells**





# GSH/GSSG-Glo™ Assay – Representative Data

After subtracting background  
(signal from empty wells)  
calculate the ratio:

$$GSH = total\ GSH - GSSG$$

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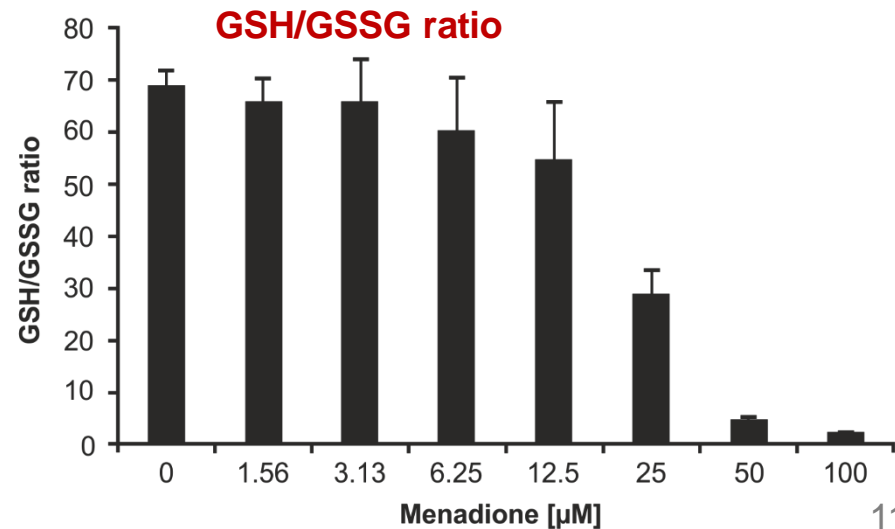
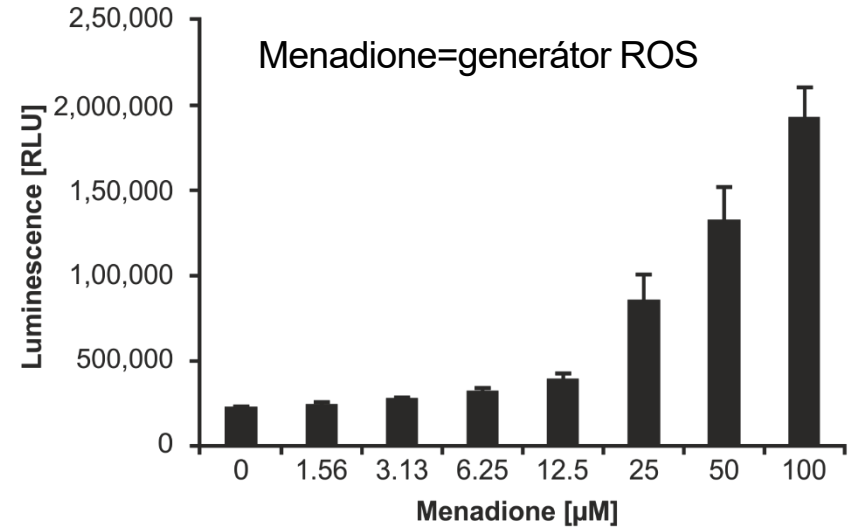

$$GSSG$$

Calculate the GSH/GSSG ratio for treated cells:

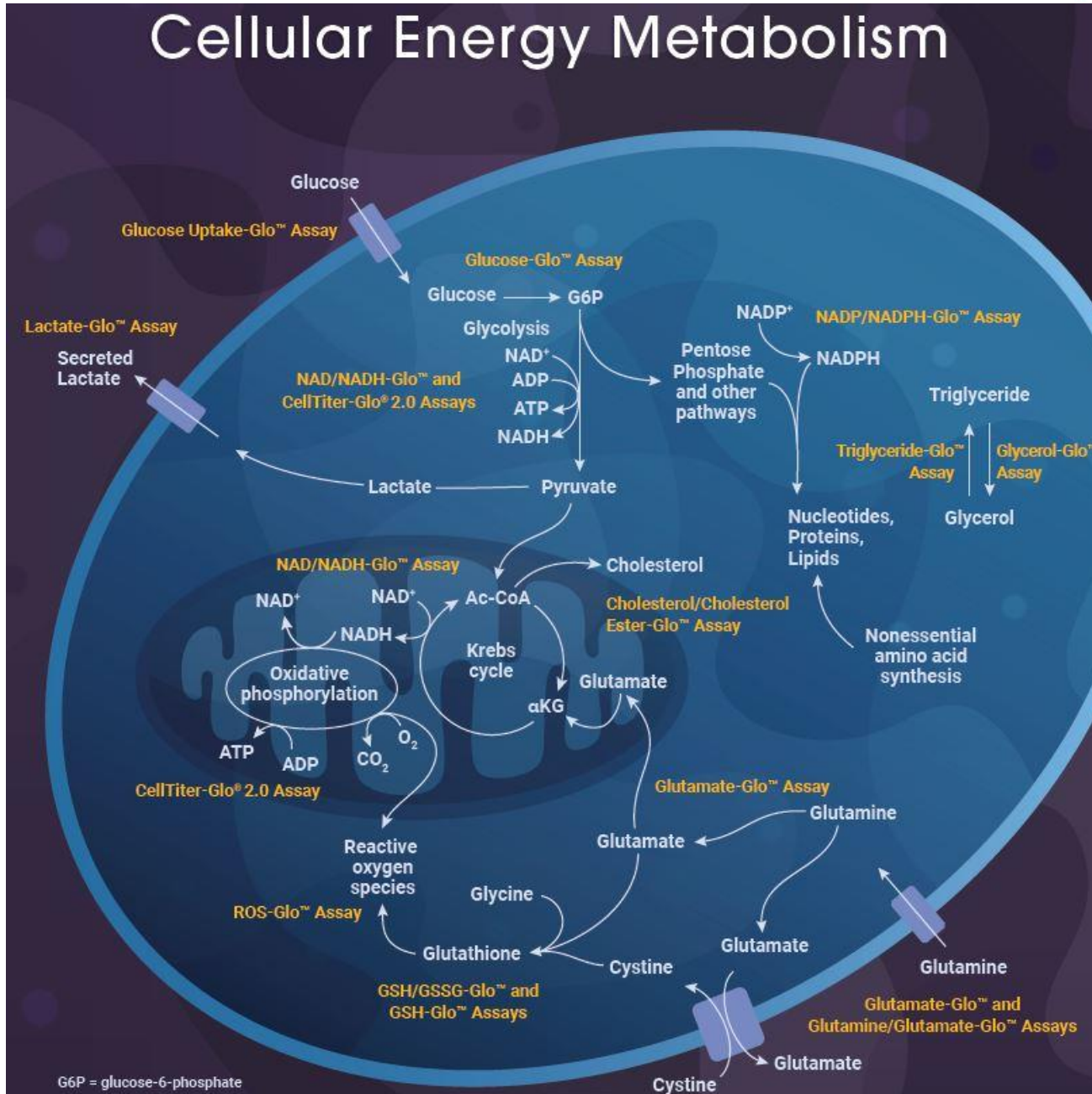
$$\frac{(Net\ treated\ total\ glutathione\ RLU - Net\ treated\ GSSG\ RLU)}{[Net\ treated\ GSSG\ RLU/2]}$$

**GSSG**

**A549 cells**



# Cellular Energy Metabolism

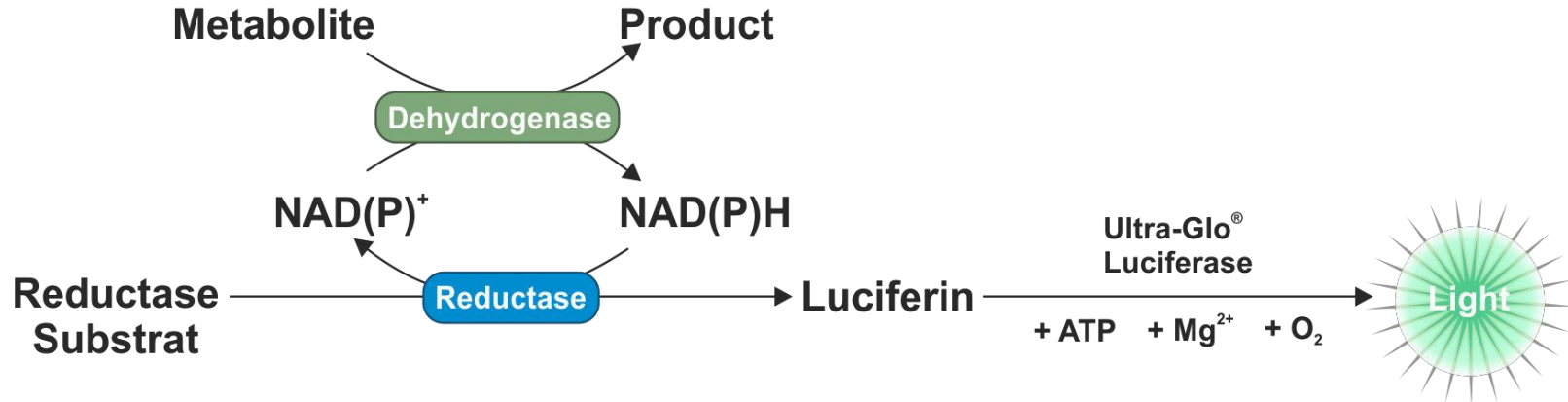


G6P = glucose-6-phosphate



# Metabolite Assays – One Reaction to Rule Them All

*Metabolite-selective dehydrogenases coupled to bioluminescent NAD(P)H detection*



*“With the aid of auxiliary enzymes nearly every substance of biological interest could be measured with a pyridine nucleotide system”* Oliver Lowry JBC (1961) 236, 2746

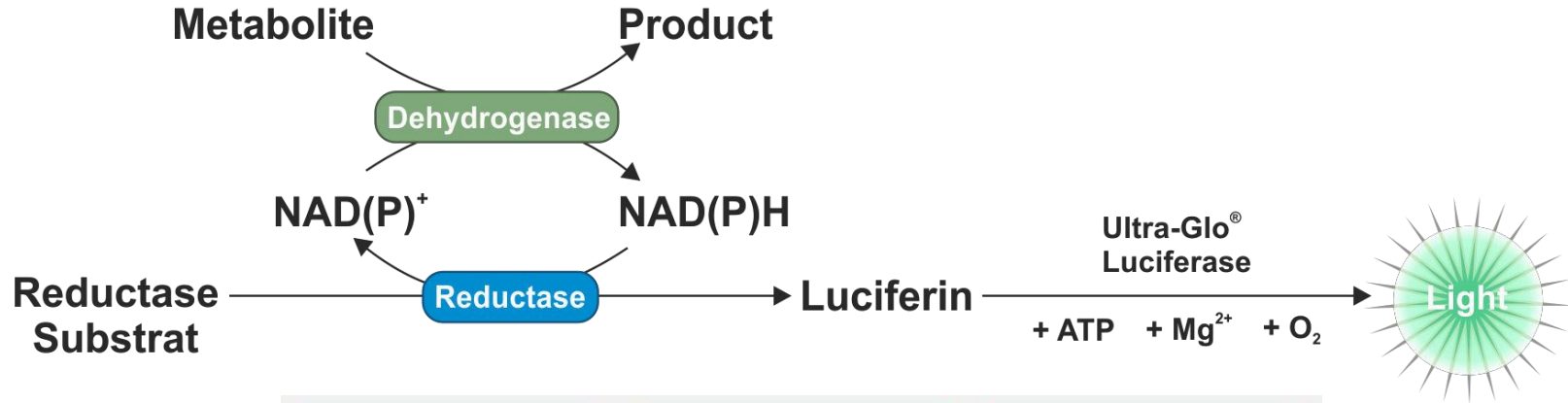
## BENEFITS

- ✓ Broad linear range of up to 3 logs (0.1 – 100 μM)
- ✓ Wide dynamic range S/B < 100
- ✓ High sensitivity, requiring only small amounts of sample
- ✓ Simplified protocol applicable to many samples types



# Metabolite Assays – One Reaction to Rule Them All

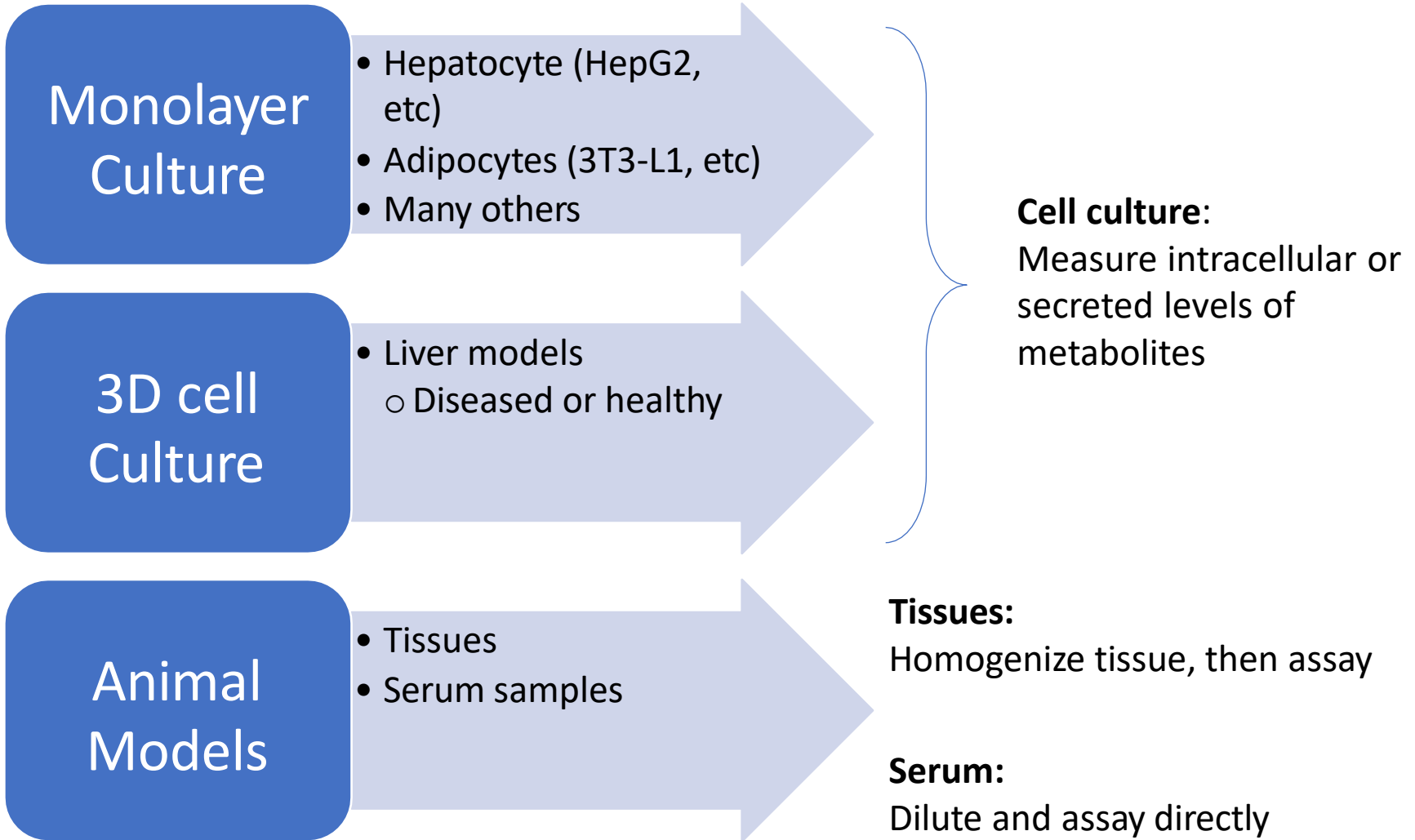
*Metabolite-selective dehydrogenases coupled to bioluminescent NAD(P)H detection*



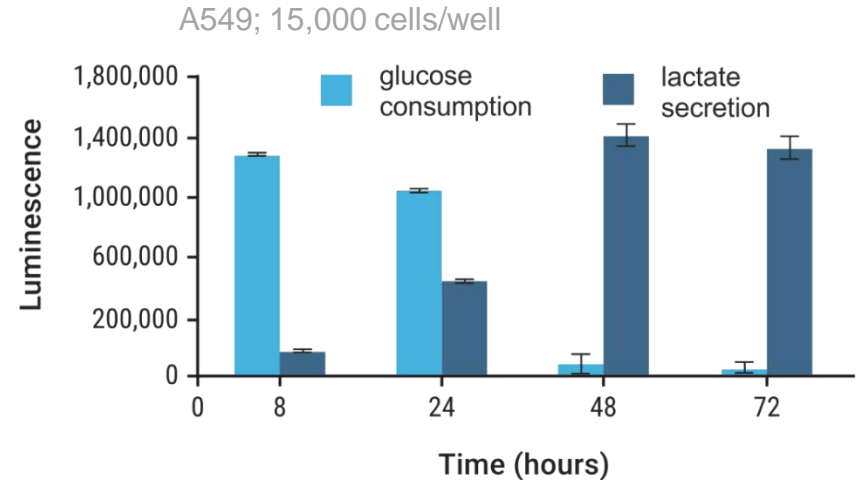
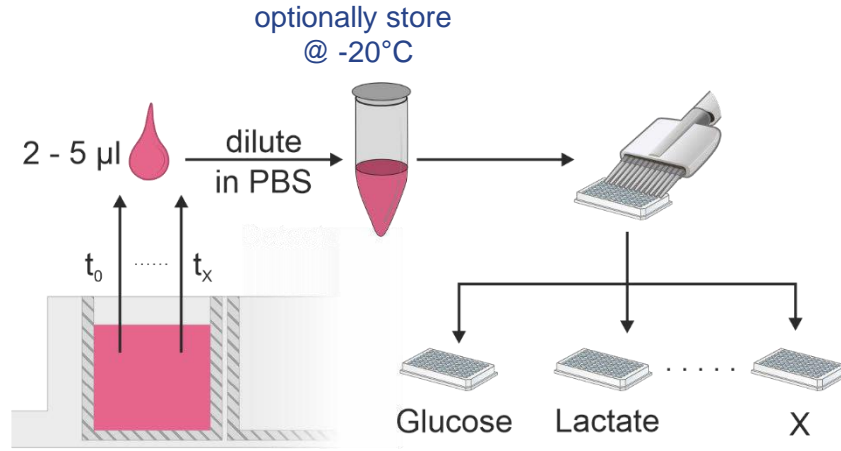
<p><b>Glucose-Glo™ Assay</b></p> <p>Easily detect glucose from a variety of biological samples.</p> <p>J6021, J6022</p>	<p><b>Lactate-Glo™ Assay</b></p> <p>Bioluminescent assay to quickly detect lactate from a variety of sample types.</p> <p>J5021, J5022</p>	<p><b>Glutamate-Glo™ Assay</b></p> <p>Quickly measure glutamate from many sample types.</p> <p>J7021, J7022</p>
<p><b>Glutamine/Glutamate-Glo™ Assay</b></p> <p>Detect glutamine and glutamate in biological samples.</p> <p>J8021, J8022</p>	<p><b>Glucose Uptake-Glo™ Assay</b></p> <p>Non-radioactive assay for measuring glucose uptake.</p> <p>J1341, J1342, J1343</p>	<p>Soon also <b>Glycogen-Glo</b></p>



# Assays are suitable for a wide range of samples



# Glucose & Lactate-Glo™ Assays



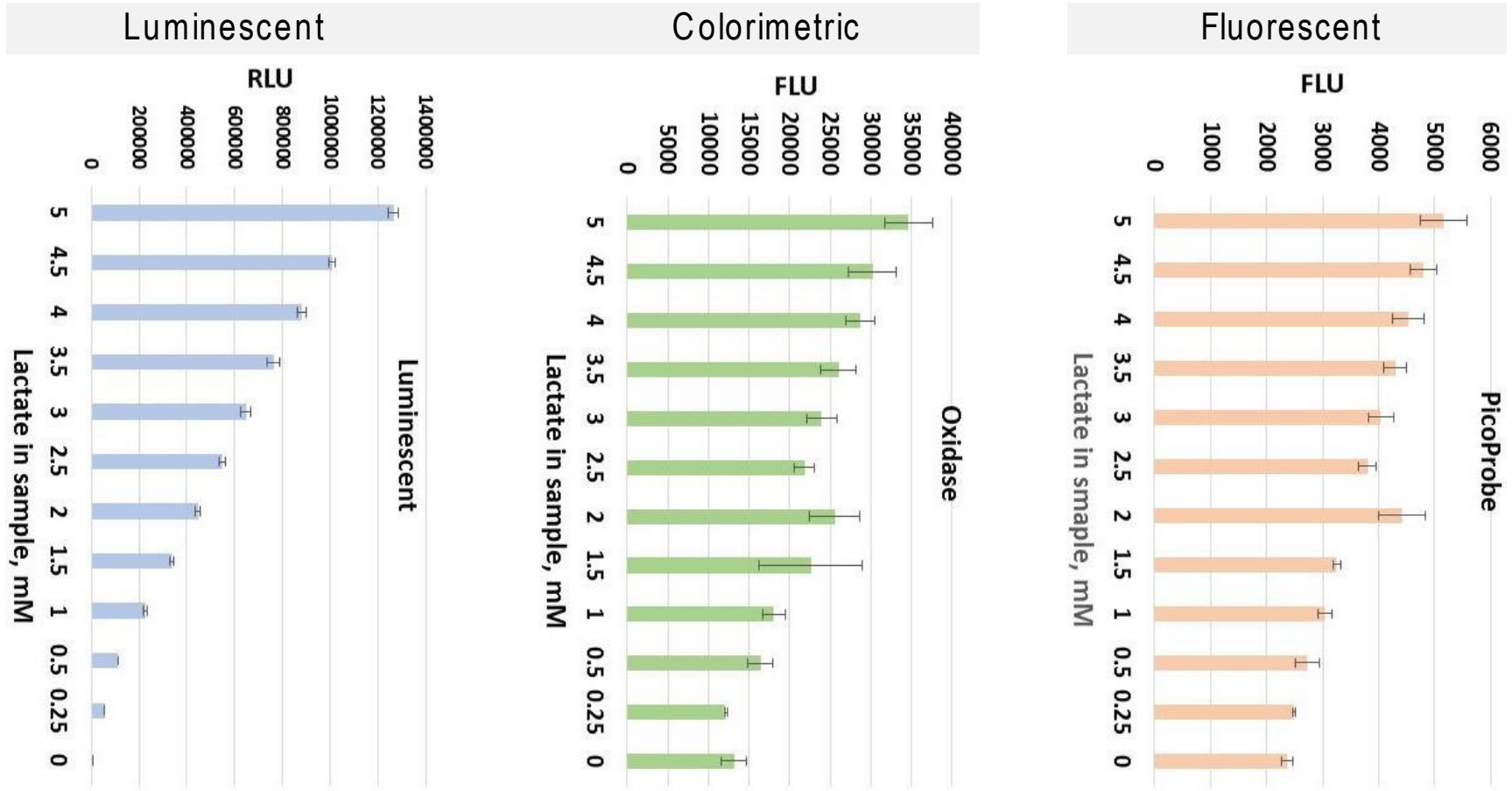
- Only 2–5 µl sample / measurement
- Multiple metabolites are easily measured
- Samples can be frozen and all measured at the end of the experiment - kinetic information
- Investigate glycolysis, a central pathway for providing energy and precursors for biosynthesis
- Metabolic profiling of cancer cells, identify vulnerabilities for anti-cancer treatment
- Determine differentiation of immunologically relevant cells, e.g. T cell activation





# Glucose & Lactate-Glo™ Assays

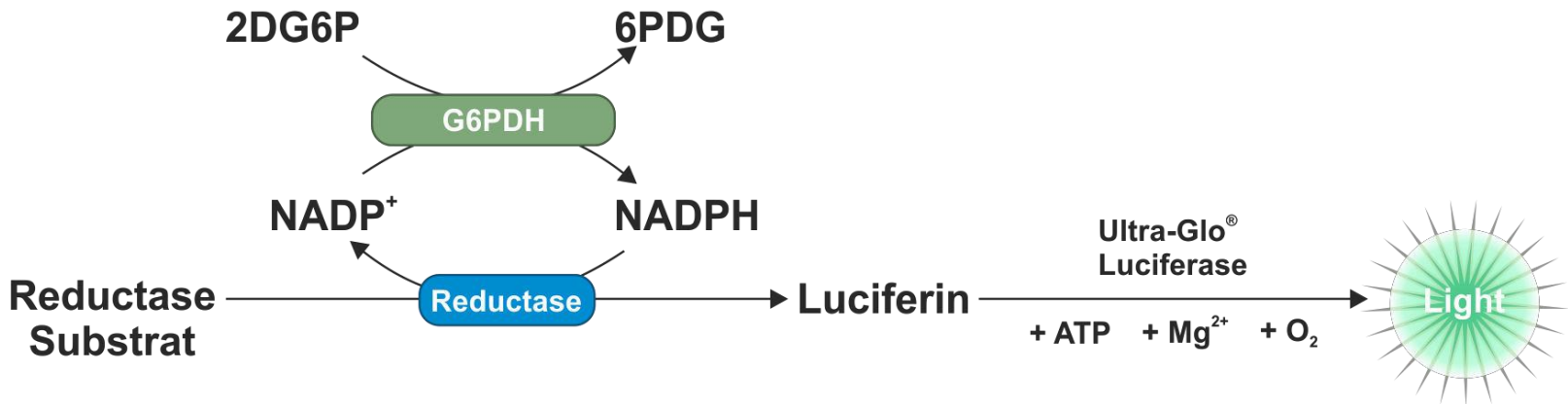
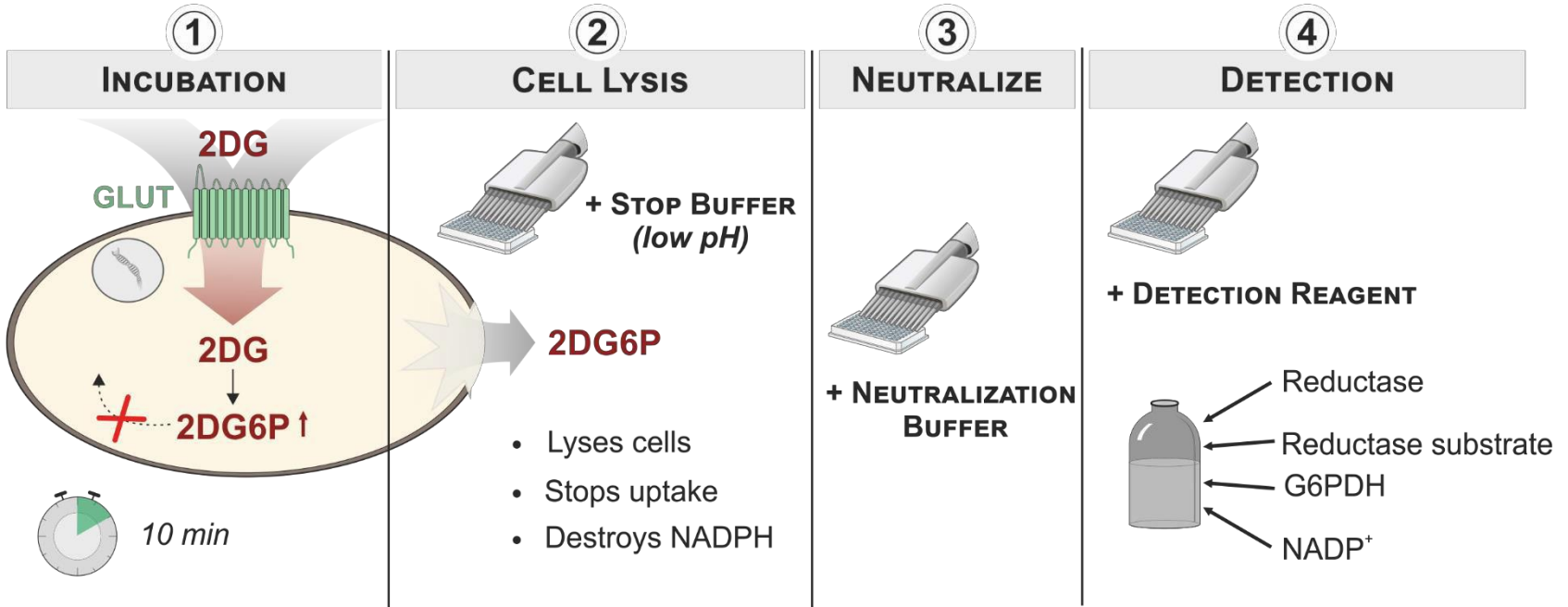
*Signal-to-Background & Dynamic Range*



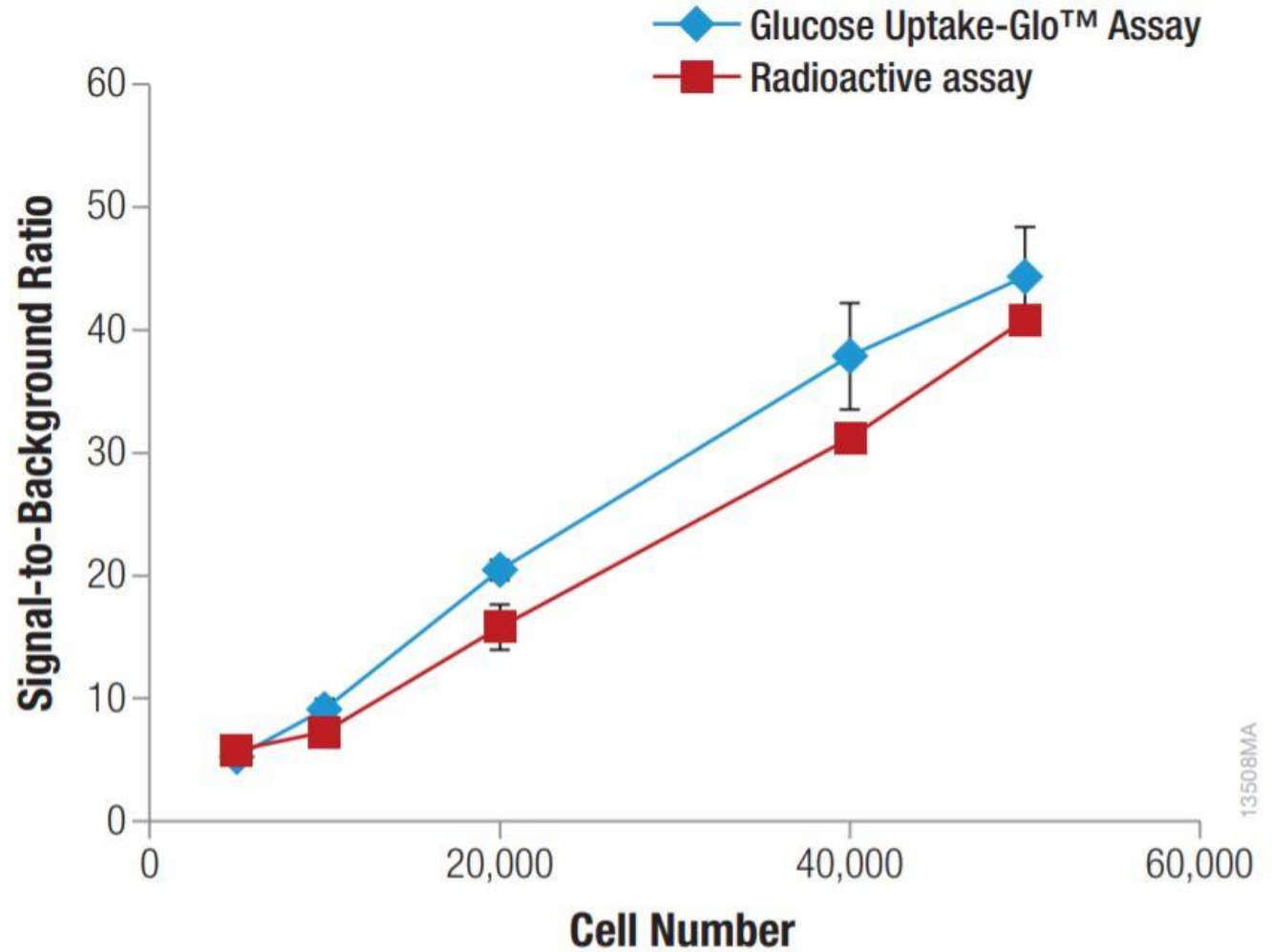
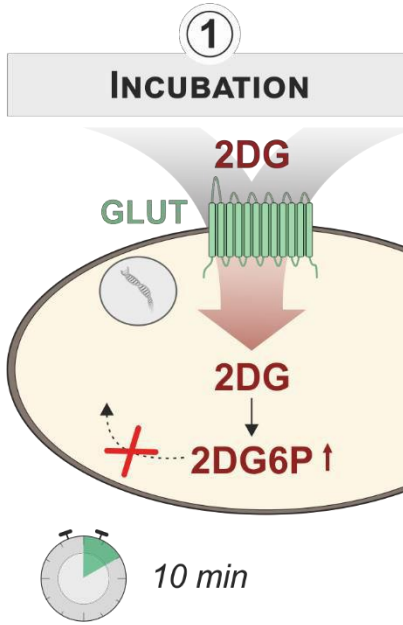
Changes in lactate secretion can be detected at lower concentrations and with higher S/B



# Glucose Uptake-Glo™ Assay – Workflow



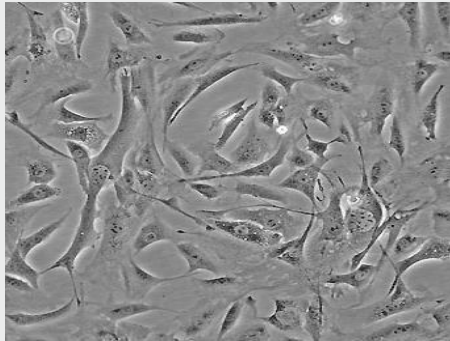
# Glucose Uptake-Glo™ Assay – As sensitive as radioactivity





# Measuring Insulin Sensitivity with Glucose Uptake-Glo™

3T3 L1-MBX cells

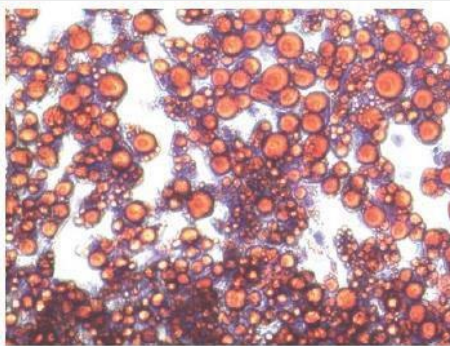


*fibroblast*

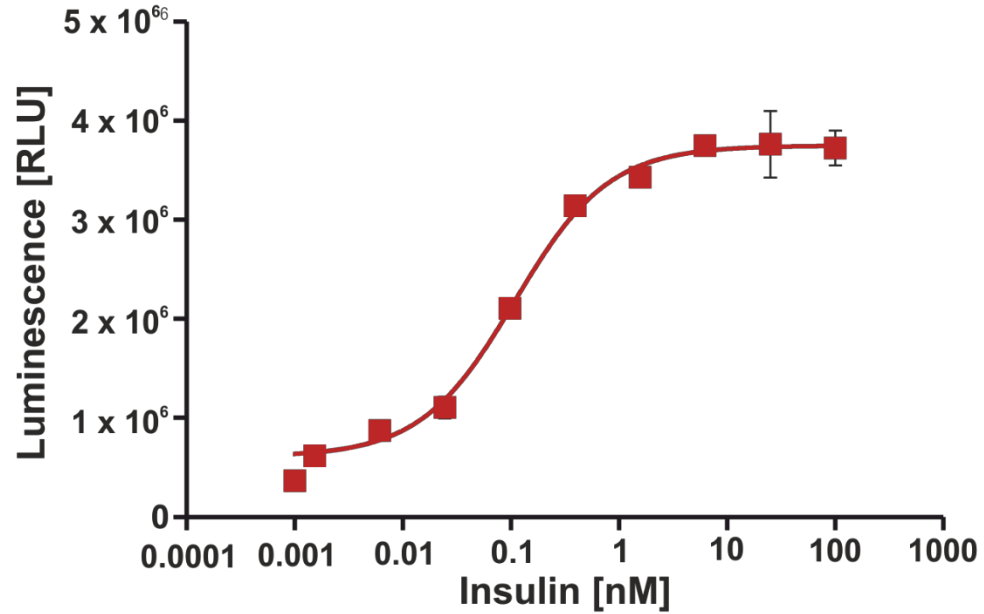
DIFFERENTIATION



*adipocyte*



oil red staining

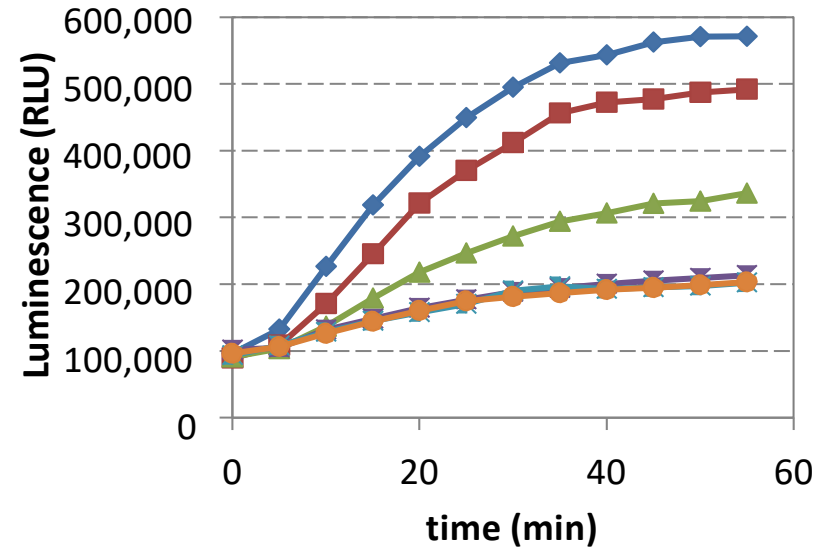
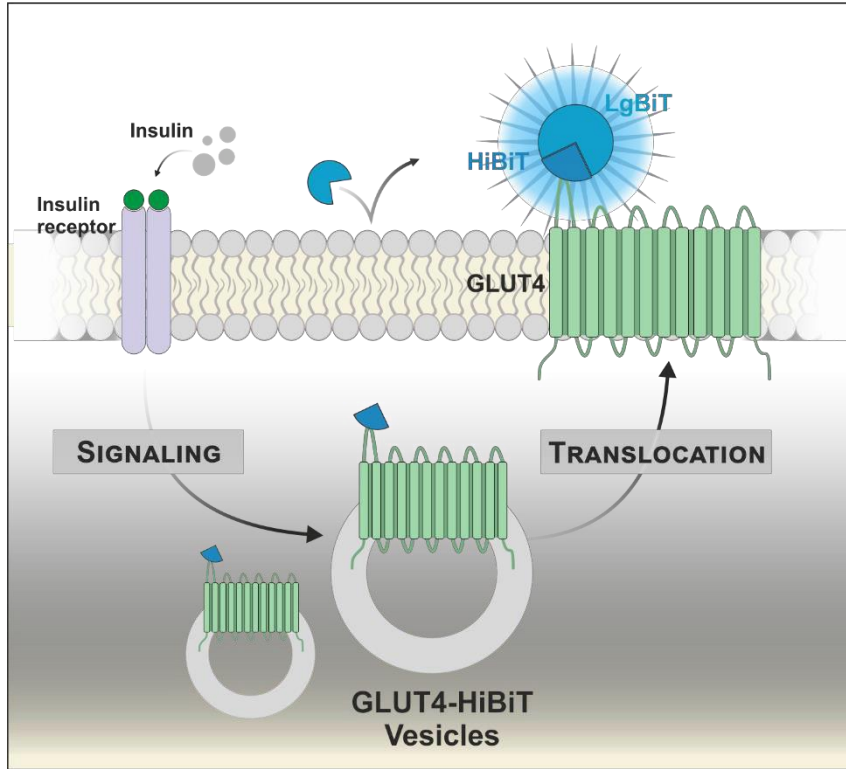


Insulin was added for 1 h in serum- and glucose-free medium.

Medium was removed and 2DG (1 mM) in PBS was added for 10 minutes.



# GLUT4-HiBiT Translocation Assay



**Insulin**

- ✦ 1 nM
- ✦ 0.1 nM
- ✦ no ins
- ✦ 1000 nM
- ✦ 100 nM
- ✦ 10 nM

- GLUT4 = insulin sensitive glucose transporter
- Stable C2C12 cells available



ADIPOSE  
TISSUE



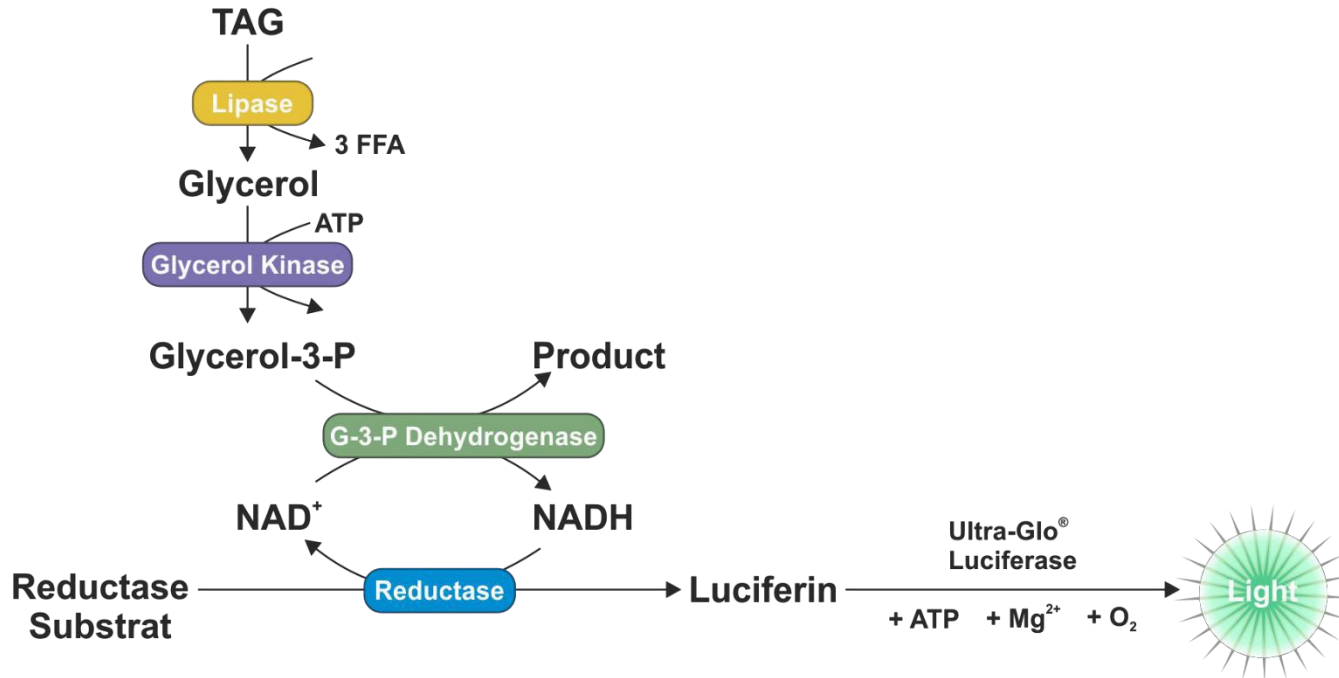
SKELETAL  
MUSCLE



MYOCARD



# Glycerol / TAG Detection Assay – Principle

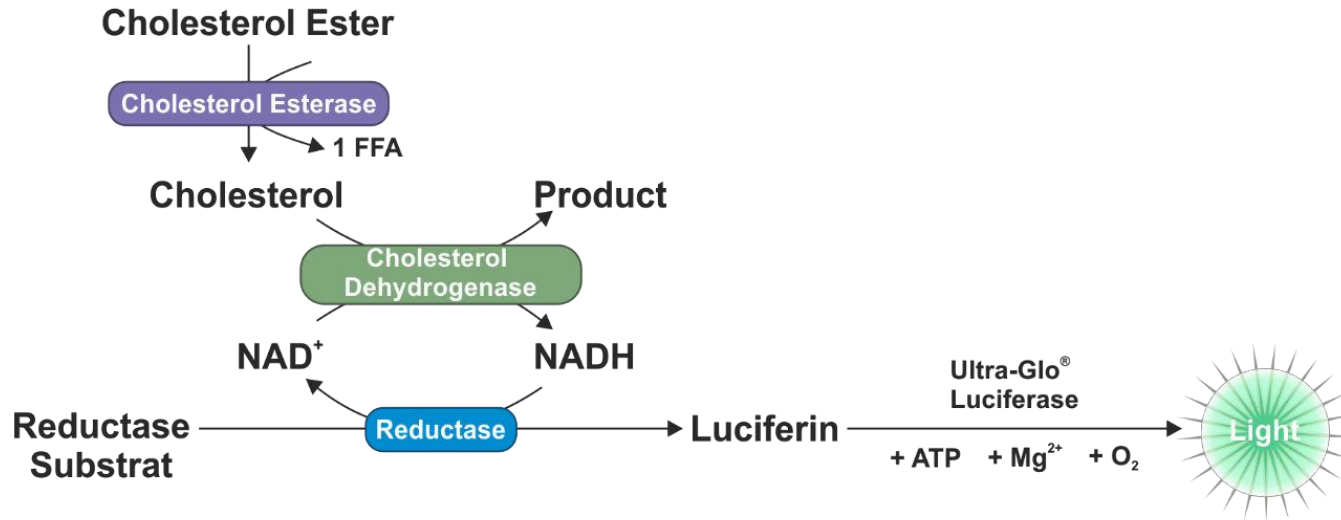


## BENEFITS

- Quantitative results
- No sample extraction steps
- Simple and fast protocol
- Bioluminescent detection mode = high sensitivity



# Cholesterol / Cholesterol Ester Detection Assay



## BENEFITS

- Quantitative results
- No sample extraction steps
- Simple and fast protocol
- Bioluminescent detection mode = high sensitivity

# GloMax plate readers



GloMax® Discover

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- ✓ Luminescence
- ✓ Fluorescence
- ✓ UV/Vis Absorbance
- ✓ BRET / FRET



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  - ✓ Shaking
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  - ✓ Fluorescence
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  - ✓ UV/Vis Absorbance
  - ✓ BRET / FRET



GloMax® Navigator

96-well

- ✓ Luminescence





# Promega is a traditional provider of molecular biology reagents and tools

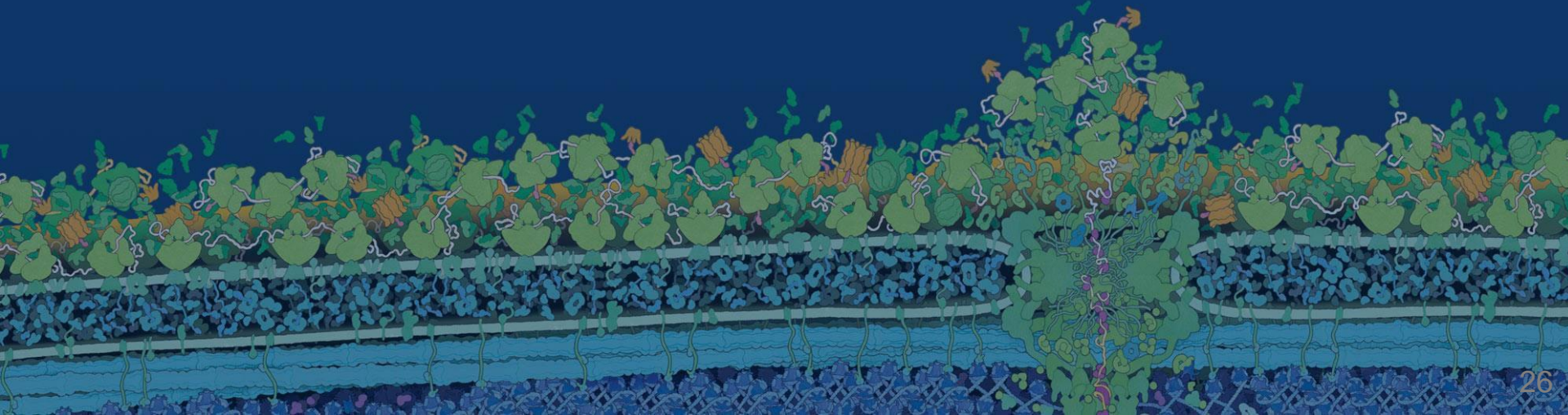
- Genomics, Proteomics, Luciferase assays, Immunoassays, Genetic identity, Capillary electrophoresis, luminometry...

- Genomics promotion throughout 2021
- **40-50% discounts** every month on one segment of the genomics portfolio
- March: Pfu polymerase
- April: Wizard SV Gel and PCR clean-up system
- Watch our website for updates



# Summary

- I showed you assays to measure ROS and redox state of cells and different metabolites.
- Ratio of reduced to oxidized glutathione is a good readout of cellular redox state.
- All metabolite detection assays work according to the same principle, just the specific dehydrogenase changes.
- Promega has good assays also for quantifying lipids.
- We also provide instruments to measure the assays we offer.
- We have a lot of molecular biology reagents, transfection reagents, cell culture media, DNA probes and oligos, small lab equipment, etc.



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- To generate your unique discount code

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KOŠÍK

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POTVRZENÍ OBJEDNÁVKY

Foto	Název	Dostupnost	Počet kusů	bez DPH / ks	s DPH / ks	Σ s DPH	Odstranit
	GSH/GSSG-Glo Assay v6611		- 1 +	14 137,00 Kč	17 105,80 Kč	17 105,80 Kč	



GSH/GSSG-Glo Assay v6611

5 dnů

- 1 +

14 137,00 Kč

17 105,80 Kč

17 105,80 Kč



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Bez DPH

2 968,80 Kč  
DPH

17 105,80 Kč  
Včetně DPH



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Spolehlivá a flexibilní purifikace nukleových kyselin



## NOVINKY



**Nový dodavatel siTOOLS – experti na RNA**

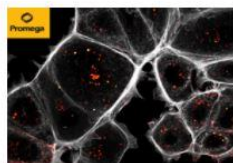
10. 3. 2021

Zahajujeme spolupráci s



**Genetický analyzátor Spectrum Compact CE**

25. 1. 2021



**Tři nastupující trendy v oblasti buněčných esejí.**

19. 1. 2021

Testování cytotoxicity,

## TWEETY



Zabýváte se RNA? Používáte siRNA a chcete se vyhnout off-target efektům? Chcete efektivně odstranit rRNA před RNA-seq? Děláte RNA pull down experimenty? Potom vás bude zajímat náš nový dodavatel @siTOOLSBiotech. Kity pro modelové i exotičtější organismy. [eastport.cz/cz/clanek/132/...](http://eastport.cz/cz/clanek/132/)



17h

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**Lonza**

