

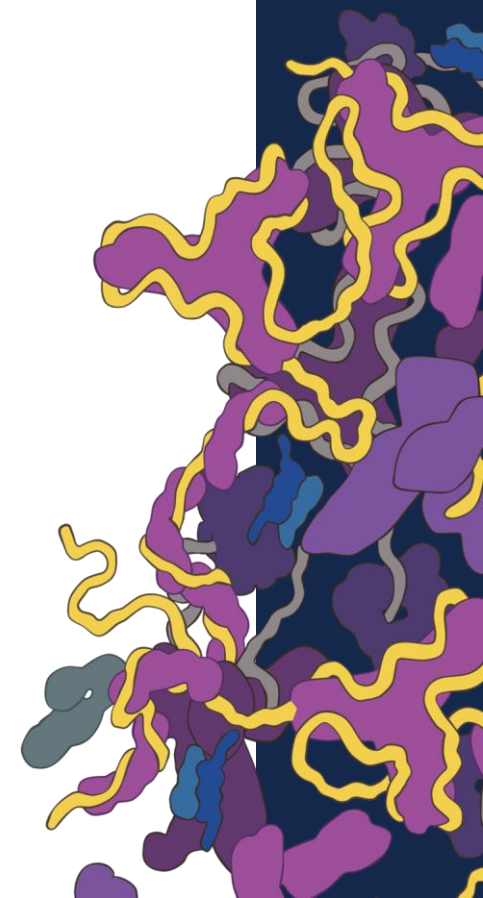
Beyond monolayers: Promega's 3D Cell Culture and Assay Solutions

Czech Republic

Dr. Kerem Yildirim

Area Manager, Central Eastern Europe

Promega Germany



Agenda of Today's Seminar

3D

= Validated for 3D use

= 3D-optimized version available

1 Introduction

2 Cell Viability

3 Cytotoxicity

→ "Cell Health"

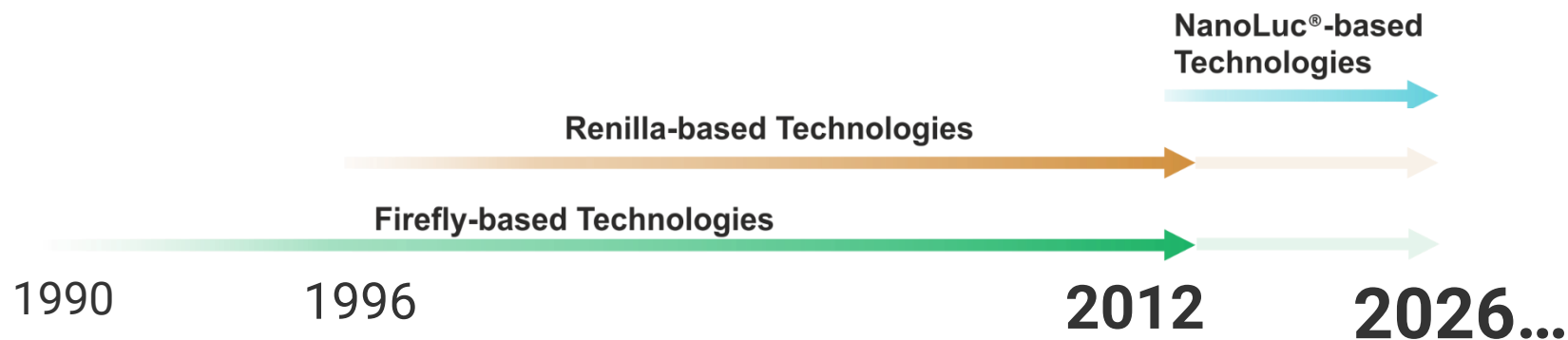
4 Proliferation

5 Apoptosis

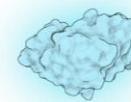
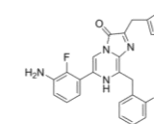
6 Metabolism

7 NanoLuc® Technology Platform

Decades of Experience with Bioluminescence



NanoLuc® luciferase

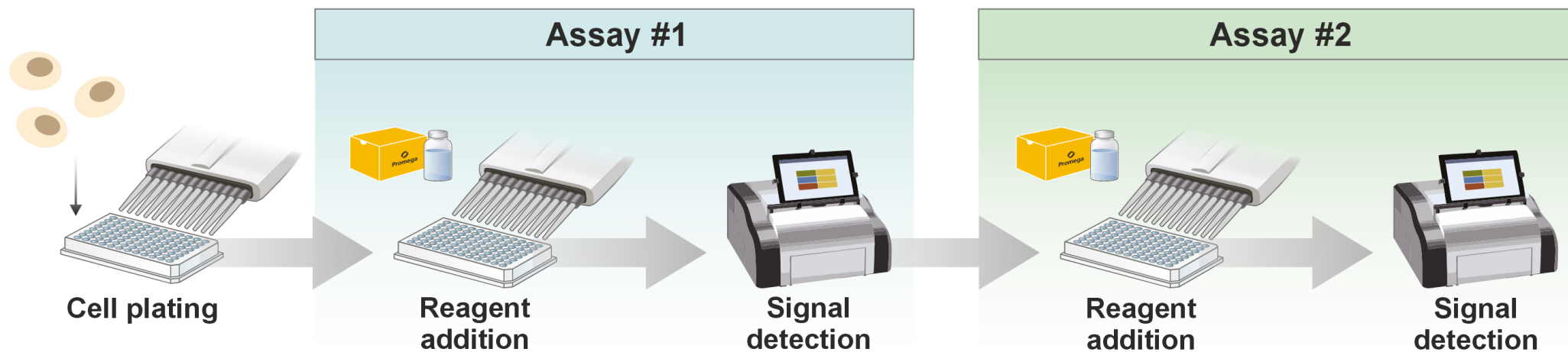


- Reporter Gene Assays
- GloSensor™ (cAMP, Protease Assays)
- GloResponse™ (Signaling Pathways)
- Rapid Response™ (Signaling Pathways)
- Cell-Health Assays
- Bioassays (ADCC, PDL1..)
- NanoBRET™ Target Engagement
- NanoBRET™ Protein:Protein Interaction
- NanoBiT® Protein:Protein Interaction
- HiBiT Protein Tagging System
- Lumit™ Immunoassays
- ...



Assay Multiplexing to Maximize Data Output

Multiplexing means measuring two or more parameters using the same sample.



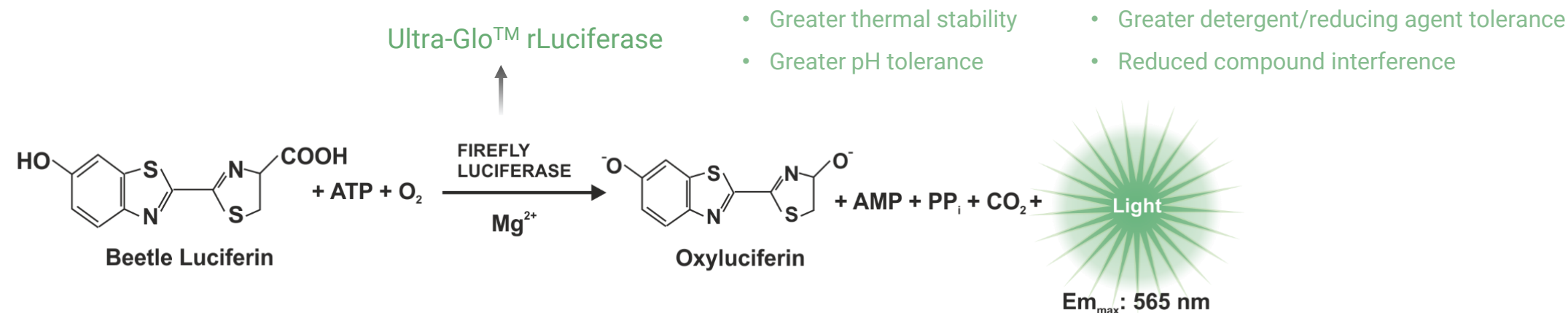
Prerequisites

- Biological & chemical compatibility of assays
- Assay signals need to be separable
- Final volume must fit with plate format

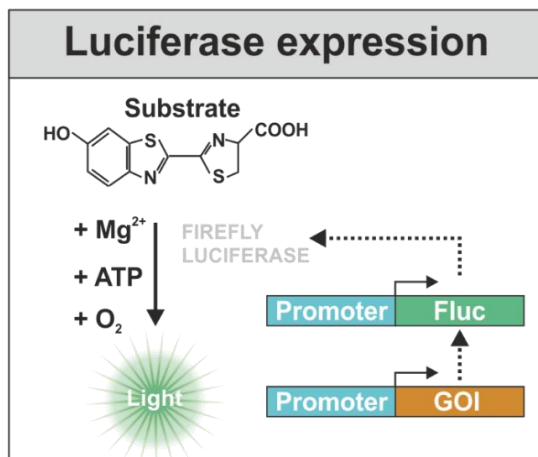
Benefits

- Two data sets with same variables
- Convenient data verification/normalization
- Cost-effective

Firefly Luciferase – A Universal Genius

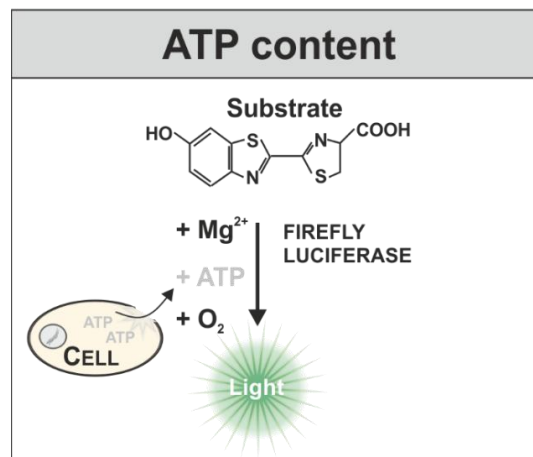


Type I

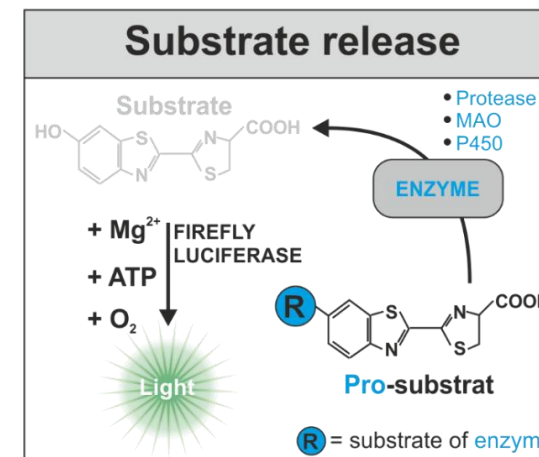


grey = limiting factor

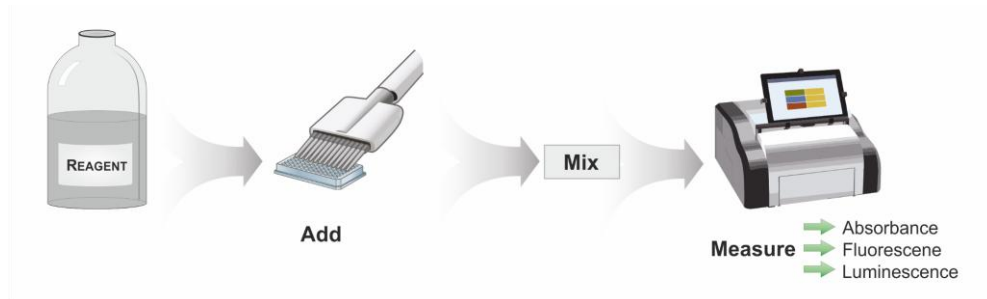
Type II



Type III

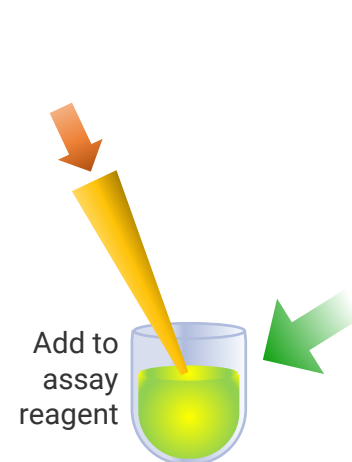


Promega's Cell-based Assay Portfolio



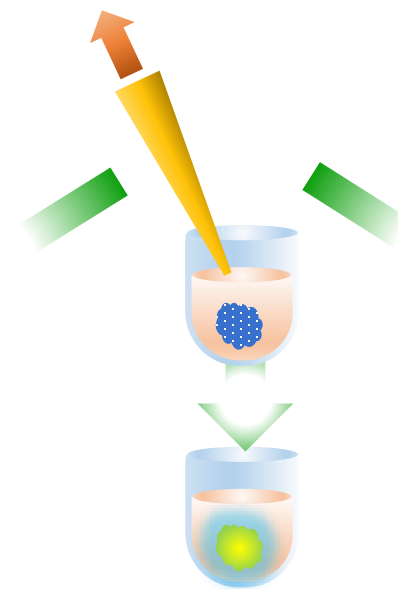
Benefits

- No cell washing
- No removal of supernatants
- Less pipetting steps
- Easy to automate
- Easy to operate
- Time saving
- HTS-compatible
- Error sources (↓)
- Reproducibility (↑)



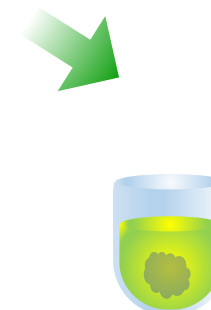
Media Sampling Non-Lytic Assays

LDH-Glo™
Cytotoxicity Assay



Live Cell Assays Non-Lytic Assays

RealTime-Glo™ MT
Cell Viability Assay



End-Point Lytic Assays

CellTiter-Glo® 2.0
Cell Viability Assay

Agenda of Today's Seminar



1 Introduction

2 Cell Viability

3 Cytotoxicity

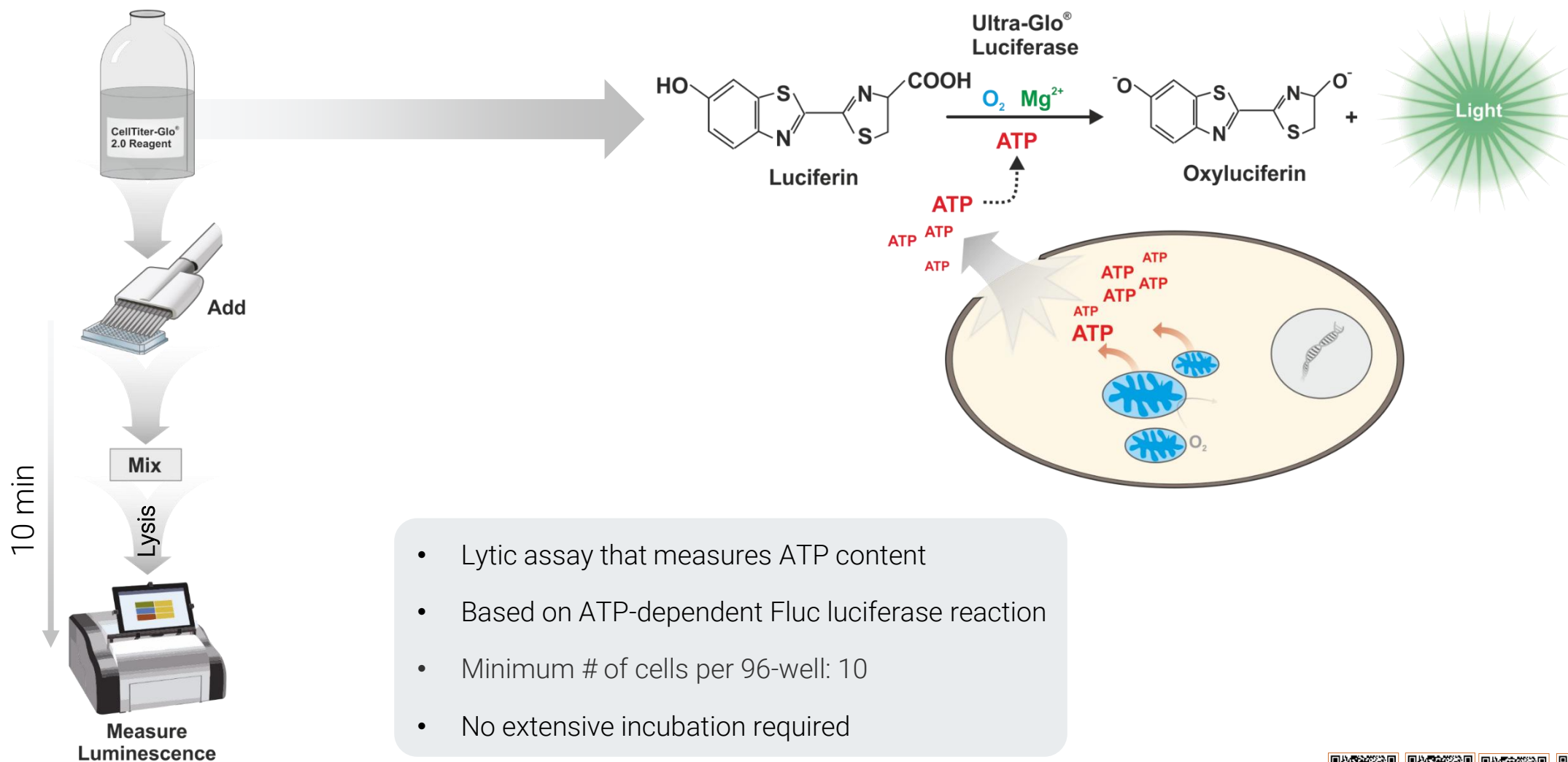
4 Proliferation

5 Apoptosis

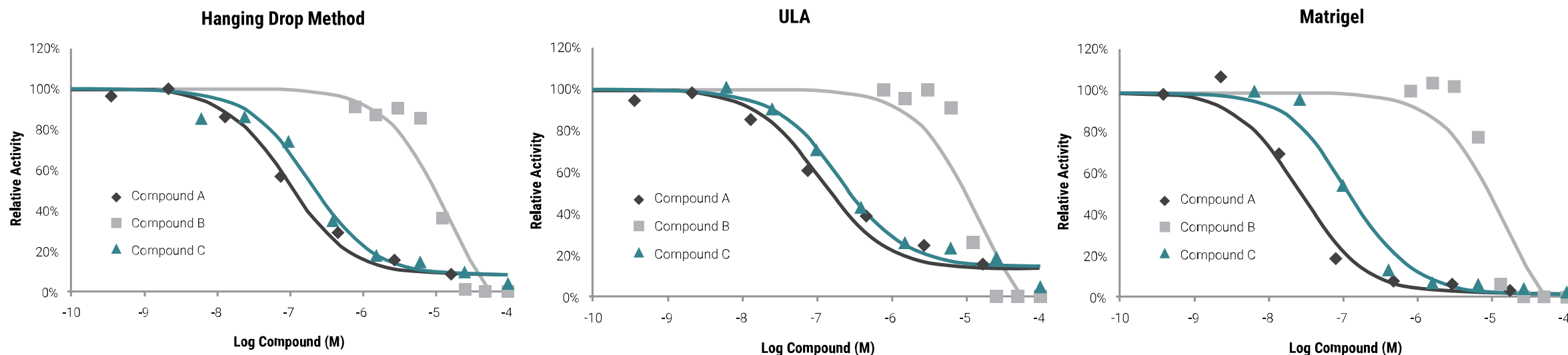
6 Metabolism

7 NanoLuc® Technology Platform

CellTiter-Glo® 3D – Promega's Gold Standard Cell Viability Assay

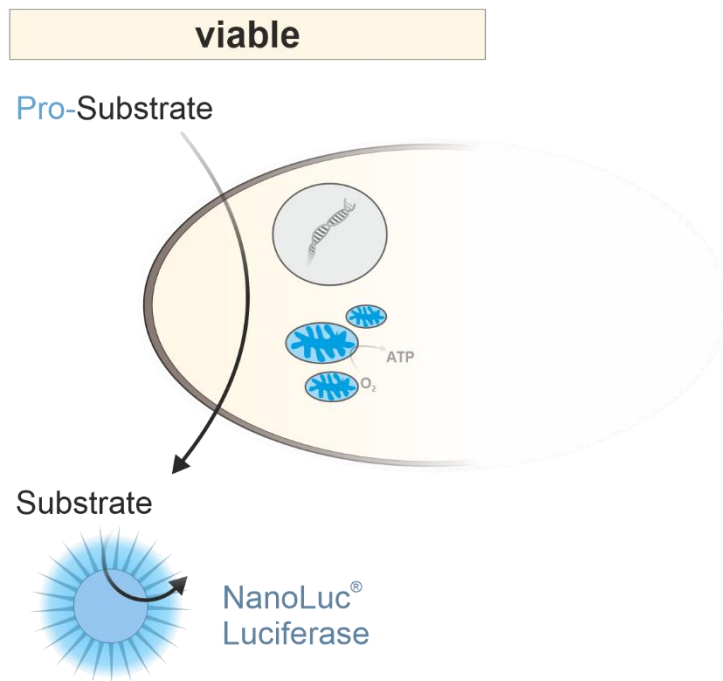


CellTiter-Glo® 3D – Promega's Gold Standard Cell Viability Assay



HCT116 colon cancer cells were seeded as follows: 400 cells in hanging-drop; 1,000 cells in ULA or Matrigel®. Microtissues were grown for 4 days, treated with compounds for 48 hours, and then assayed with the CellTiter-Glo® 3D Reagent. Luminescence was recorded at 30 minutes.

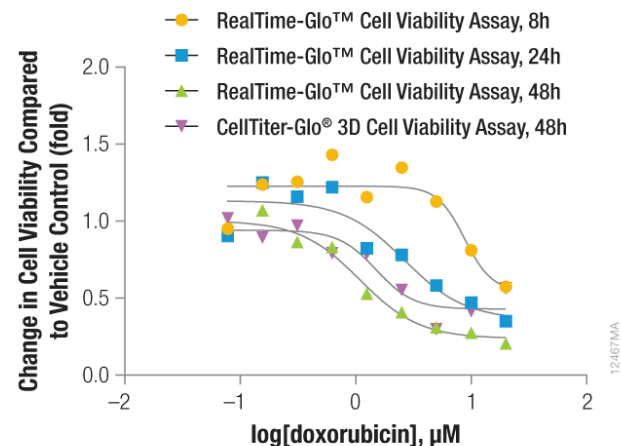
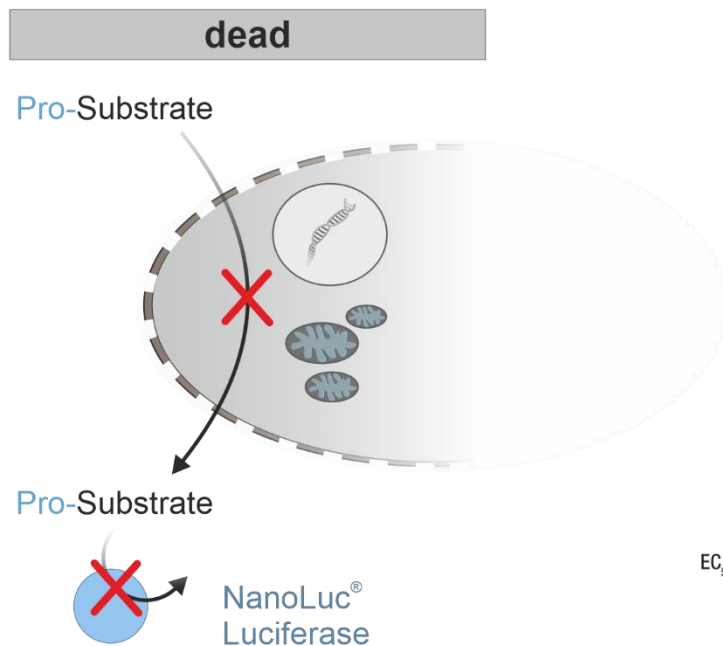
RealTime-Glo™ MT Cell Viability Assay



- No toxicity
- Supports time course analysis (≤ 72 h)
- Downstream sample processing possible (e.g., nucleic acid extraction)
- Multiplexing-compatible

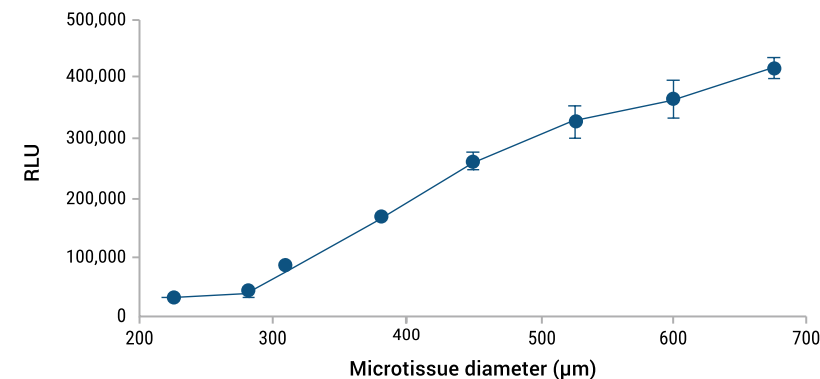


RealTime-Glo™ MT Cell Viability Assay



	RealTime-Glo™ Assay at 8 hours	RealTime-Glo™ Assay at 24 hours	RealTime-Glo™ Assay at 48 hours	CellTiter-Glo® Assay at 48 hours
EC ₅₀ Value (μM)	8.77	2.65	1.09	1.50

HCT116 colon cancer spheroids were grown for 4 days in a 96-well hanging drop plate



HCT116 spheroids of increasing size were grown for 4 days in a 96-well hanging drop plate

- No toxicity
- Supports time course analysis (≤ 72 h)
- Downstream sample processing possible (e.g., nucleic acid extraction)
- Multiplexing-compatible



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4 Proliferation

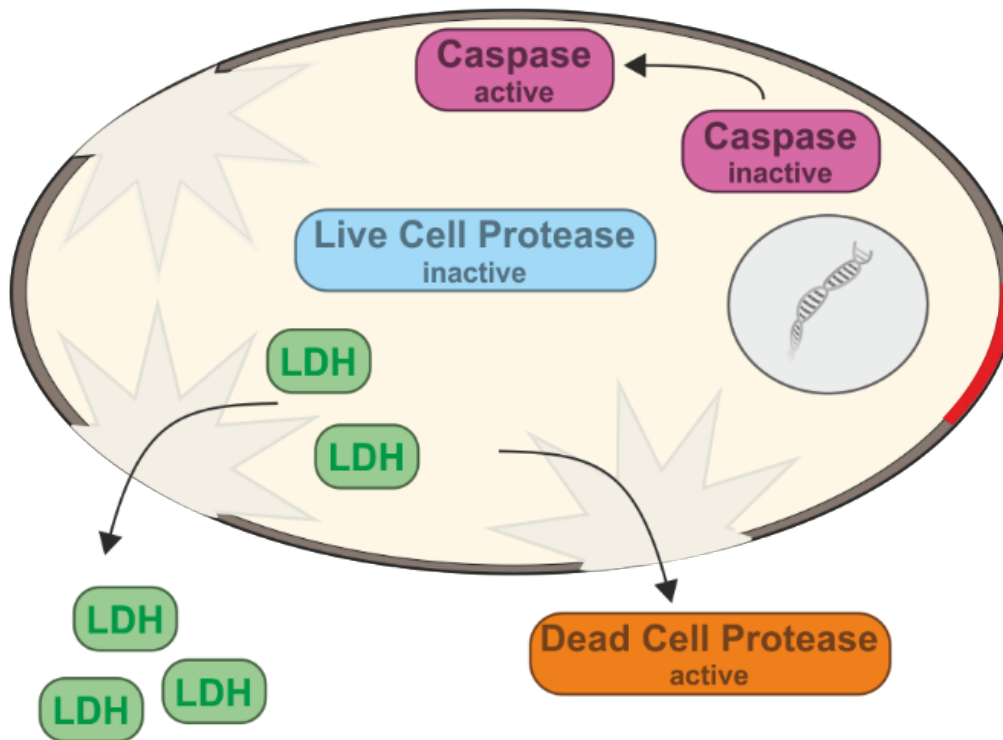
5 Apoptosis

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7 NanoLuc® Technology Platform

Biomarkers of Cytotoxicity

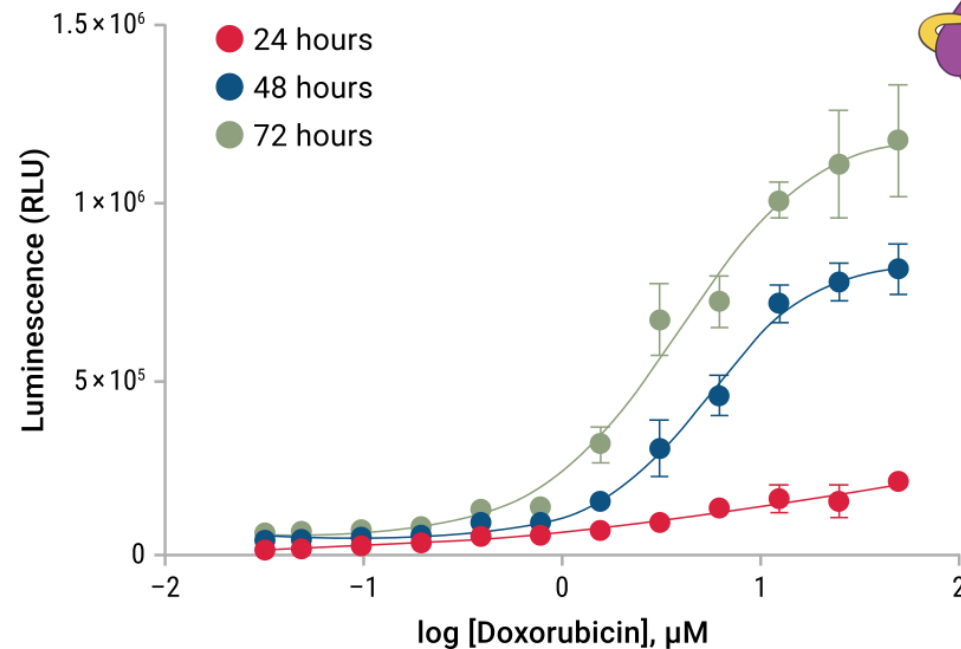
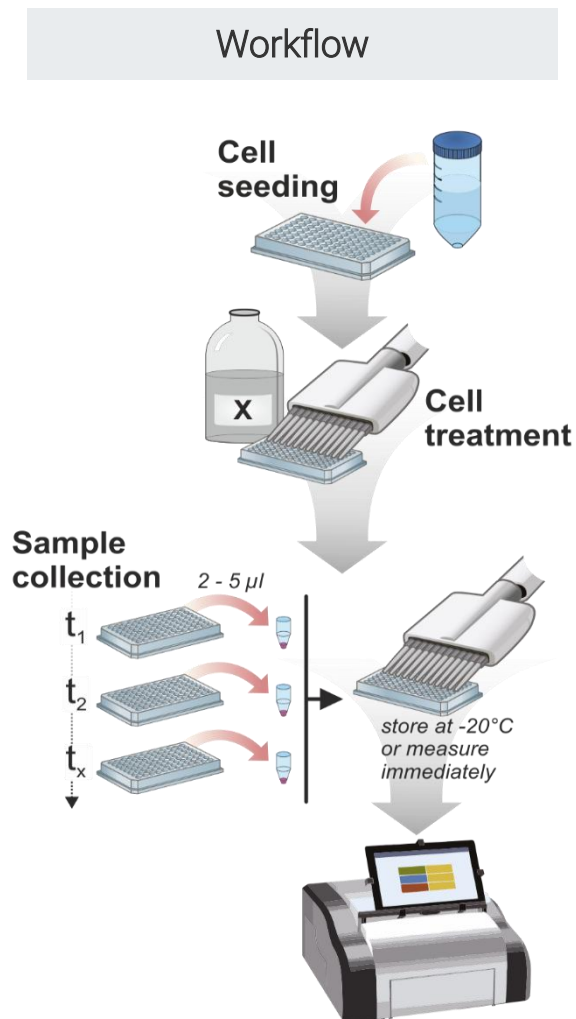
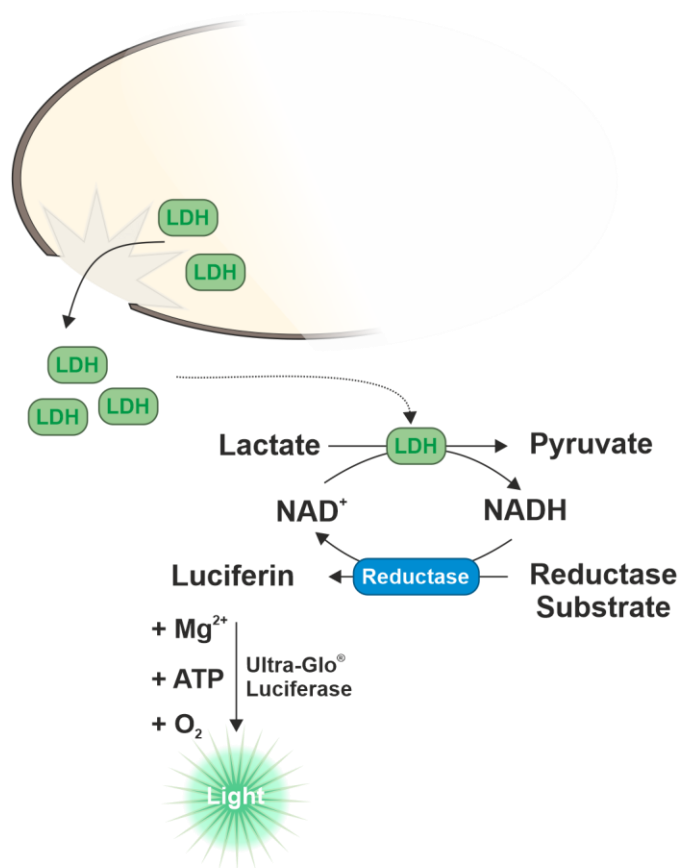
Cytotoxicity assays determine the relative amount of dead cells within a population and allow to conclude on the causal mechanism of cell death (e.g. apoptosis and necrosis).



BIOMARKER

- ① Membrane integrity
- ② LDH release
- ③ Protease activity
- ④ Membrane composition
- ⑤ ATP & NADH content ↓

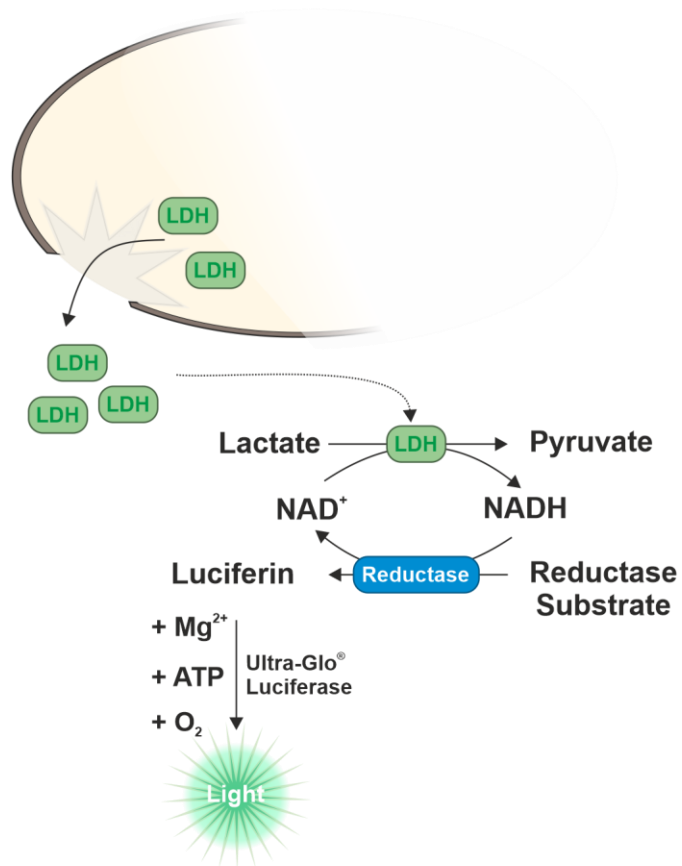
LDH-Glo™ Cytotoxicity Assay



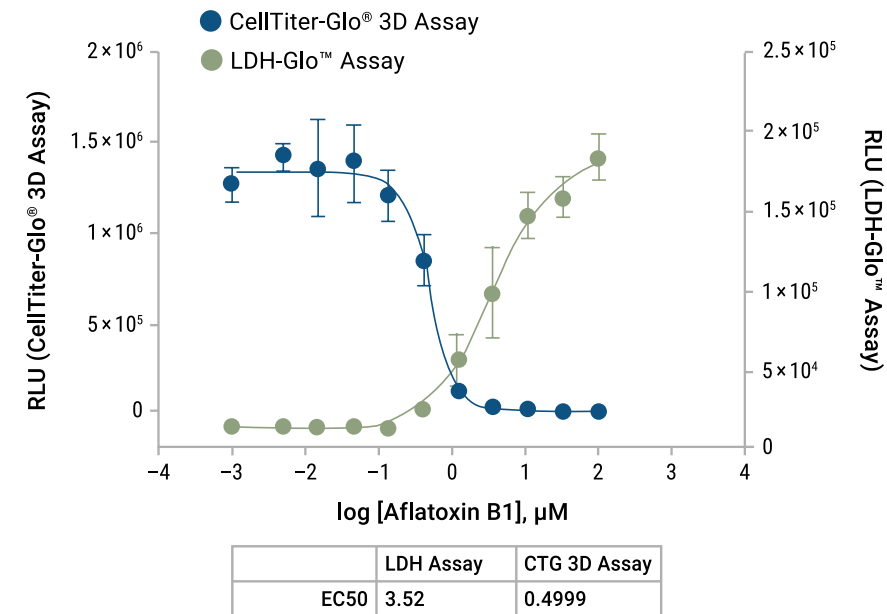
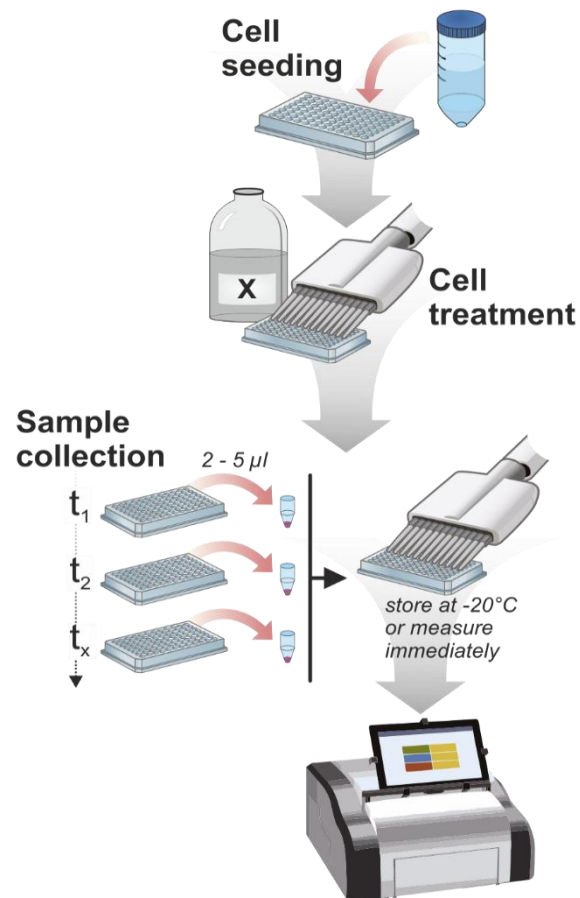
	24 hours	48 hours	72 hours
EC50	17.09	5.474	3.844

HCT116 cells were grown as spheroids in 384-well ULA plates (Corning) and treated with doxorubicin

LDH-Glo™ Cytotoxicity Assay

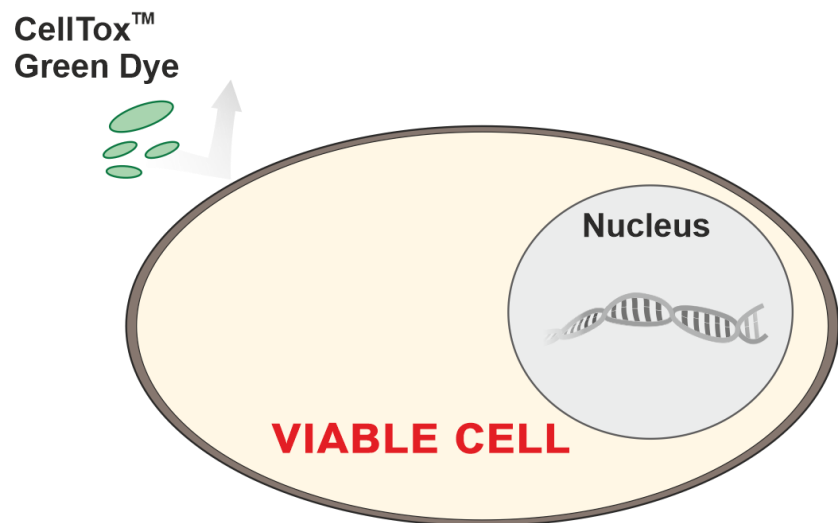


Workflow

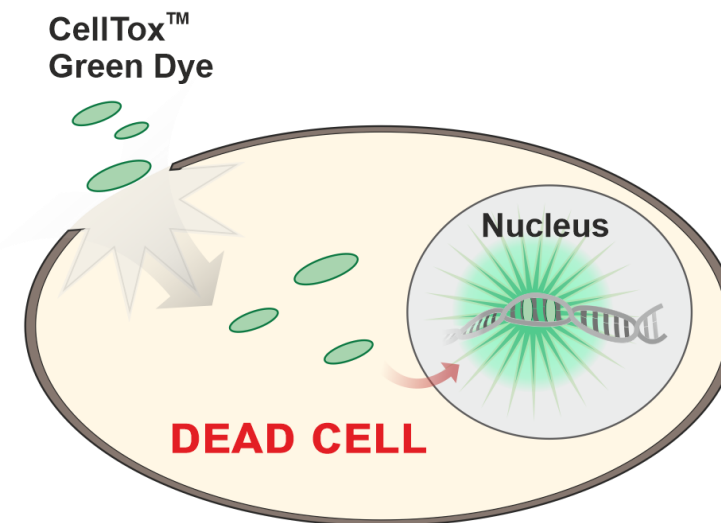


Human liver microtissue spheroids were treated with aflatoxin B1 for 48 hours.

CellTox™ Green Cytotoxicity Assay



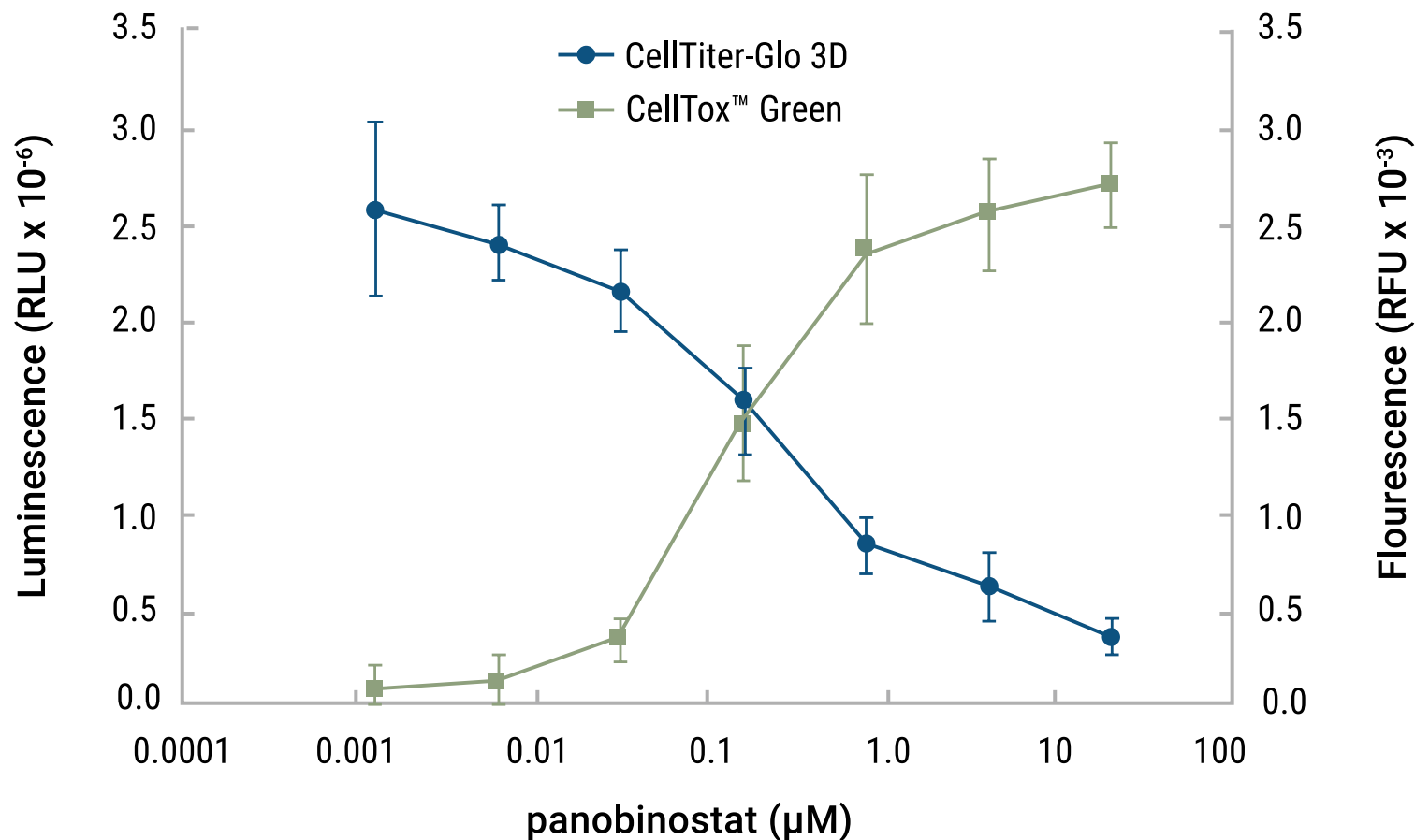
Excluded dye yields
no increase in fluorescence



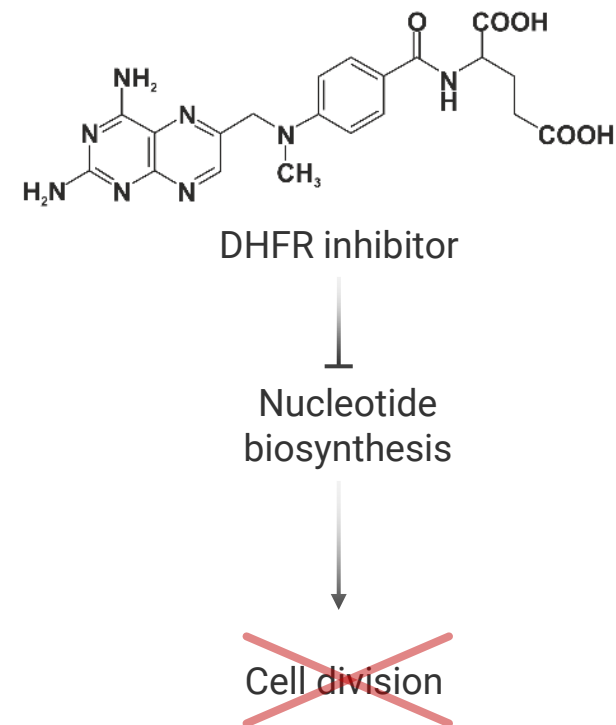
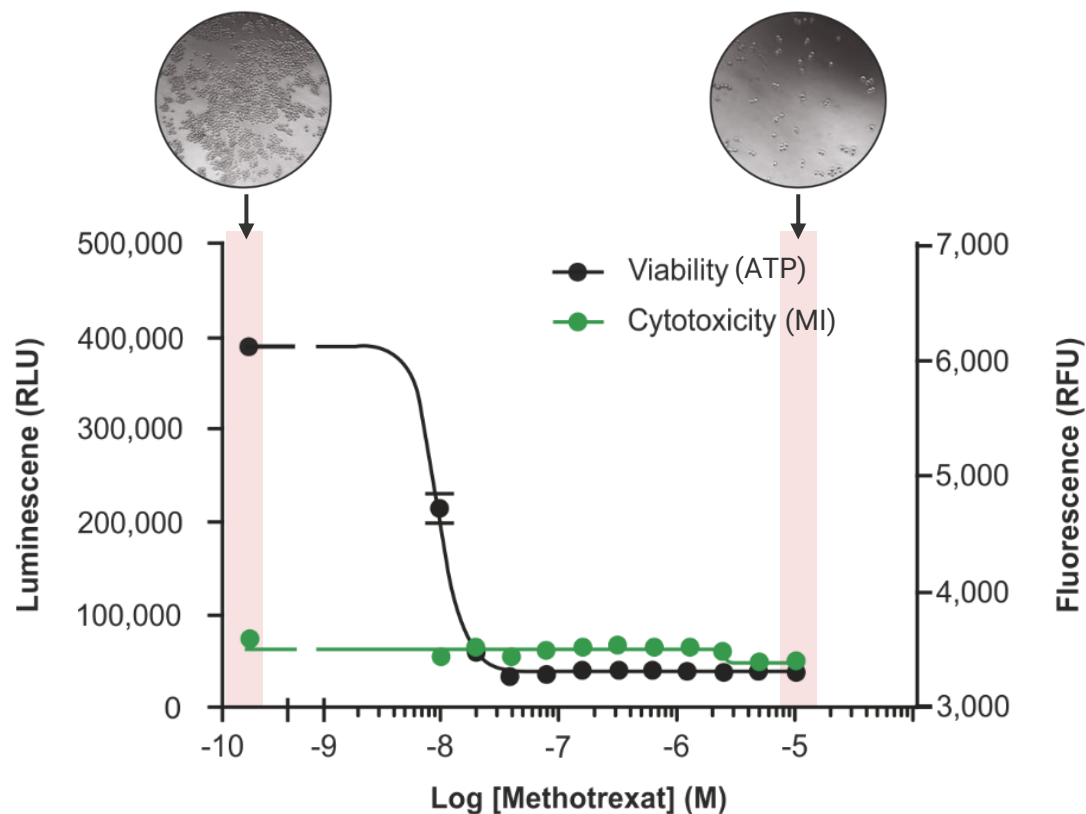
Penetrated dye yields
increase in fluorescence

- No toxicity
- Minimal membrane permeability
- Supports time course analysis (≤ 72 h)
- Multiplexing-compatible

CellTox™ Green Cytotoxicity Assay



Increasing Confidence in Data Interpretation by Multiplexing



- Assay multiplexing is a valuable tool to verify experimental conclusions
- Combining orthogonal readouts improves clarity on cytotoxic vs. cytostatic effects

Agenda of Today's Seminar



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4 Proliferation

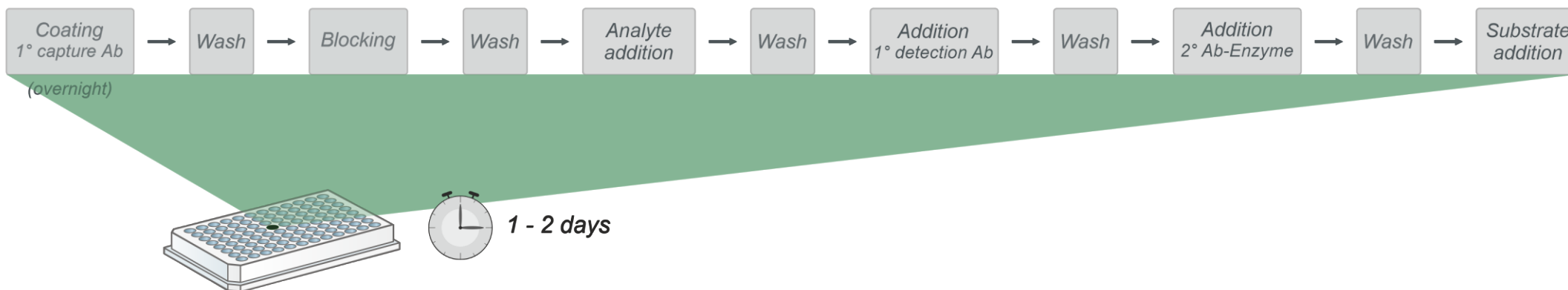
5 Apoptosis

6 Metabolism

7 NanoLuc® Technology Platform

Lumit® Immunoassay vs. ELISA

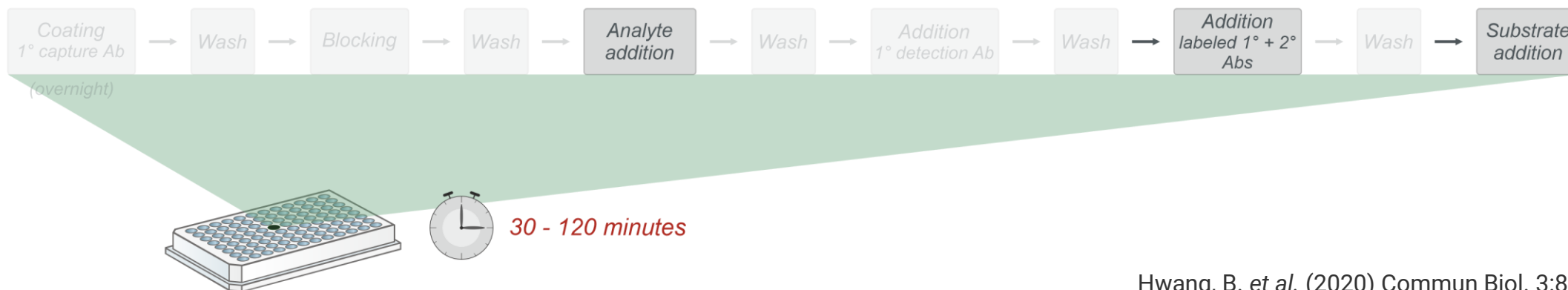
Traditional ELISA Workflow



- Traditional ELISA is a heterogenous multistep process involving several wash / incubation steps

Lumit® Immunoassay vs. ELISA

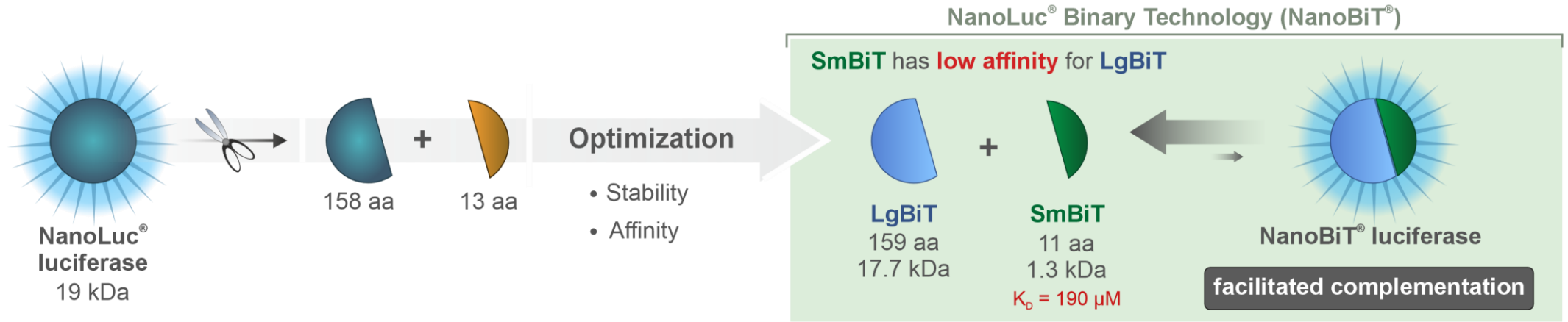
Lumit® Immunoassay Workflow



Hwang, B. et al. (2020) Commun Biol. 3:8

- Traditional ELISA is a heterogenous multistep process involving several wash / incubation steps
- Lumit® Immunoassays
 - ✓ Easy workflow with short assay time (30 – 120 min)
 - ✓ High sensitivity (low number of cells)
 - ✓ Broad dynamic range (3 – 4 logs)
 - ✓ Flexible formats (96- or 384-well)
 - ✓ Homogenous and HTS compatible
 - ✓ No specialized instrumentation required

Lumit® Immunoassay Principle



Complementation facilitated through ... (direct) protein:protein interaction

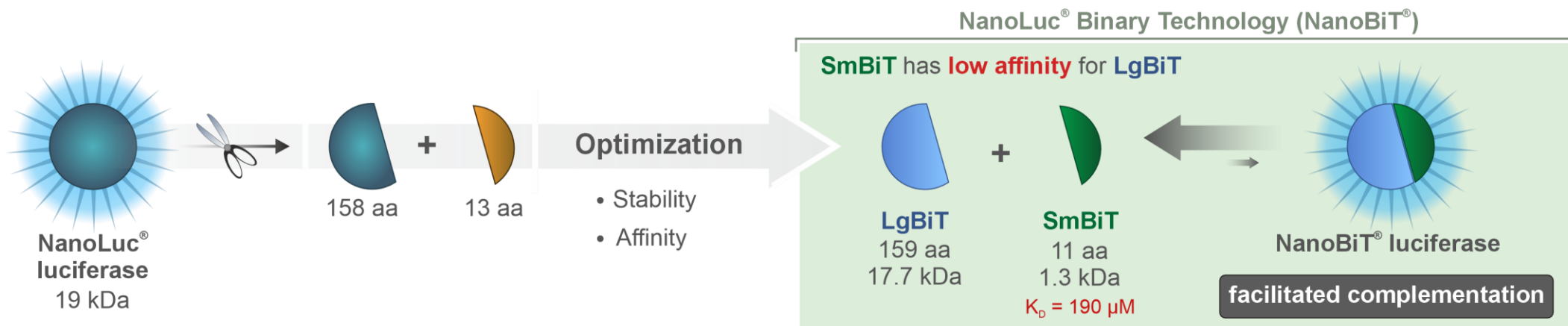


NanoBiT® PPI System

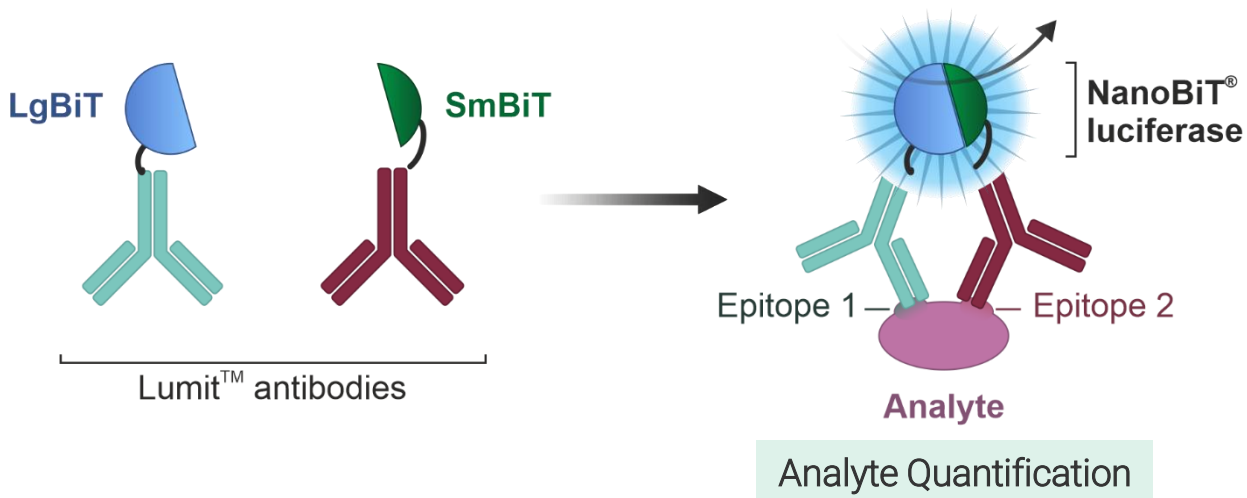
Dixon et al. 2016,
ACS Chemical Biology



Lumit® Immunoassay Principle



Complementation facilitated through ... (indirect) Ab:Ab “interaction”

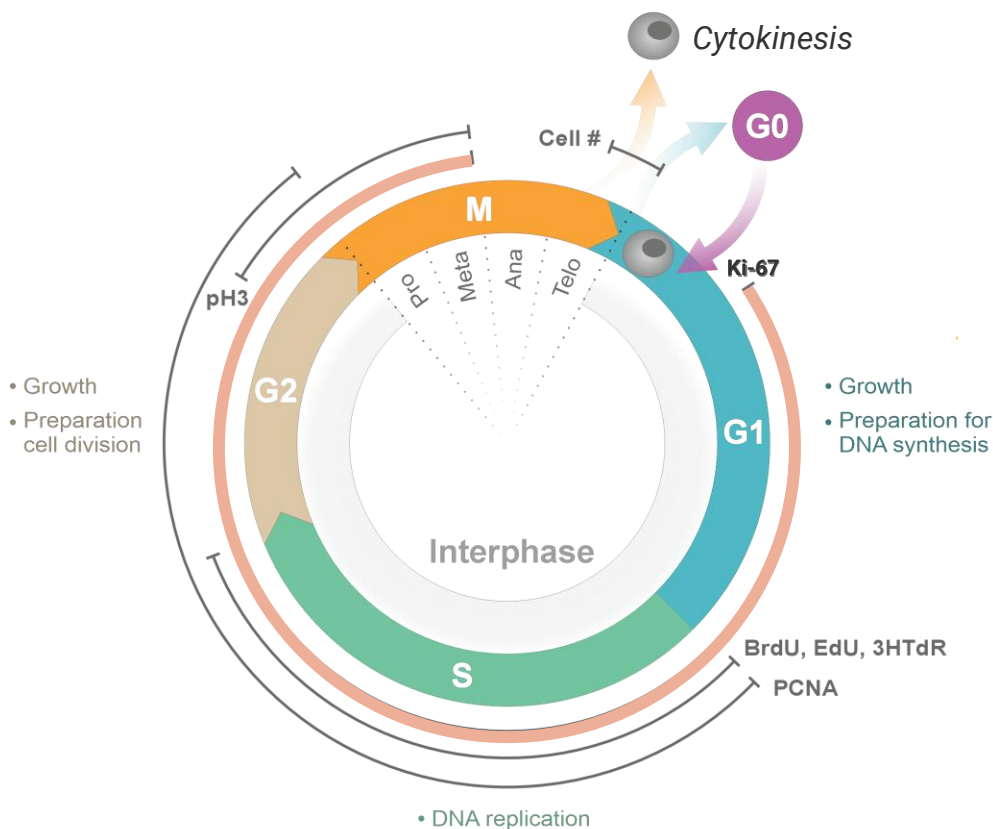


Lumit® Immunoassays

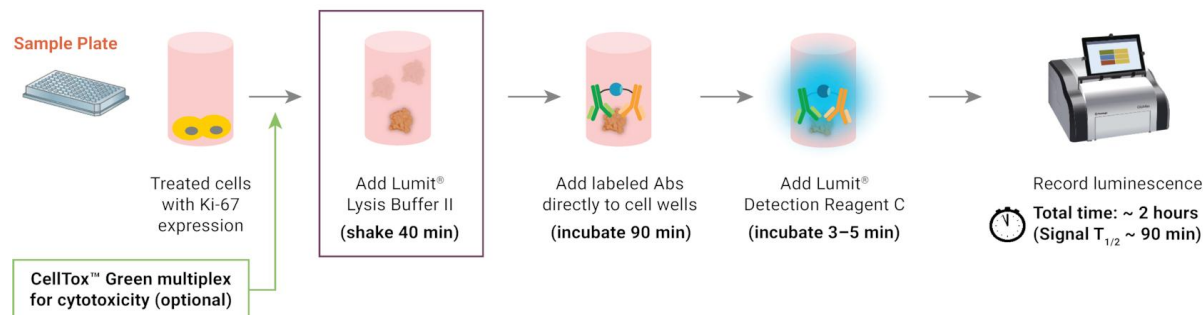
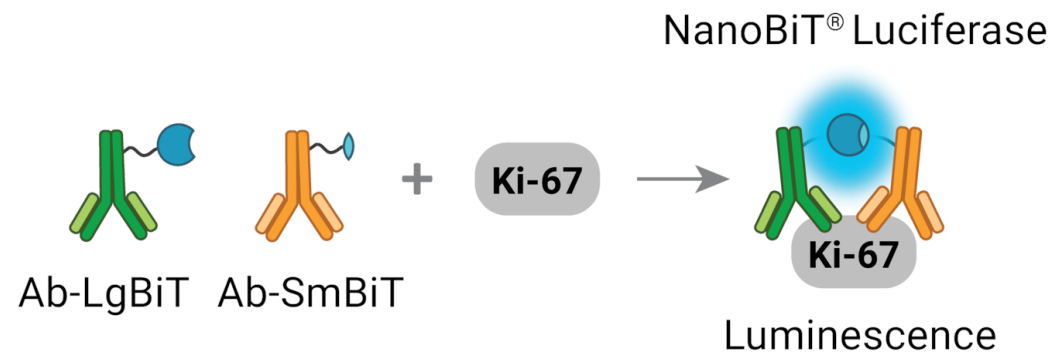
Hwang *et al.* 2020,
Commun Biol.

- IL-1 β , IL-2, -4, -6, -8, -10, -12, -17A, -18*, TNF- α , IFN- β , IFN- γ , VEGF, HMGB1, insulin, glucagon, p24, Ki-67, C1q, IgG, dsRNA*
- (Phospho-)proteins
- Fc receptor binding
- cAMP, cGAMP, cGMP

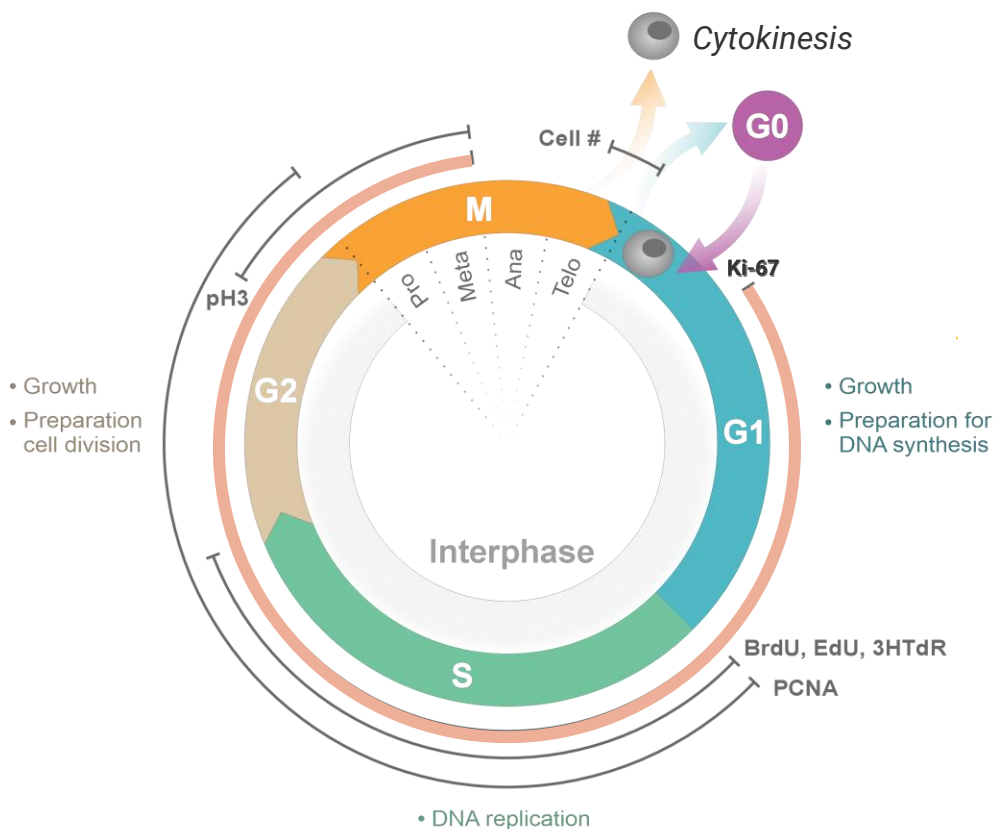
Lumit® Cell Proliferation Assay (Human Ki-67)



- Expressed in proliferating cells
 - Expressed in G1, S, G2 and M cell cycle phases
 - Ramps up from G1 until peaks early in M phase
- Absent in resting, non-dividing cells (G0) (quiescent, senescent, or terminally differentiated)

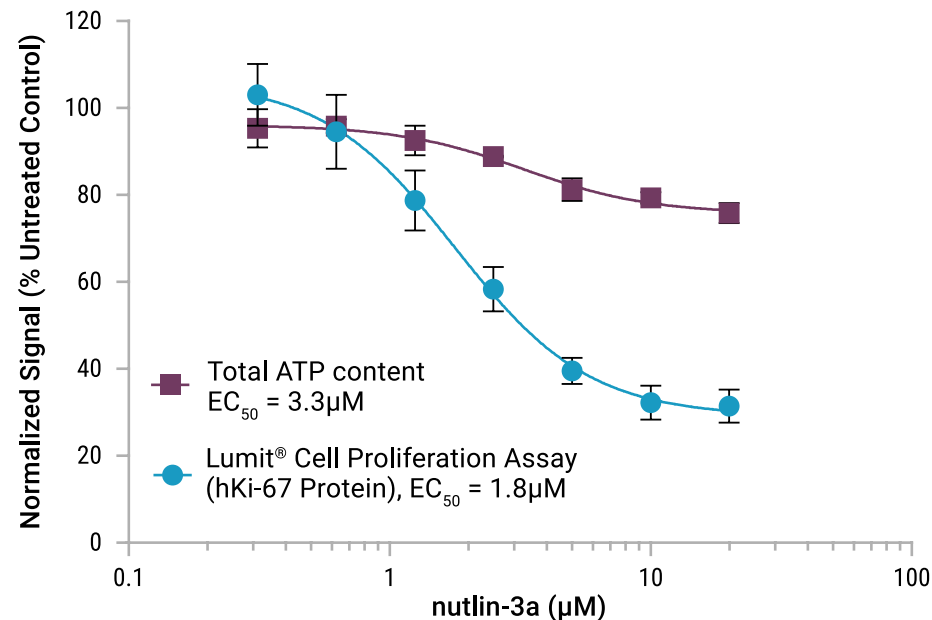


Lumit® Cell Proliferation Assay (Human Ki-67)



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 - Expressed in G1, S, G2 and M cell cycle phases
 - Ramps up from G1 until peaks early in M phase
- Absent in resting, non-dividing cells (G0) (quiescent, senescent, or terminally differentiated)

HCT116 3D Spheroids
hKi-67 Protein vs Total ATP Content



HCT116 spheroids were formed in 96-well ULA round-bottom plates (SBio PrimeSurface) and treated with nutlin-3a for 24 hours.

Agenda of Today's Seminar



1 Introduction

2 Cell Viability

3 Cytotoxicity

4 Proliferation

5 Apoptosis

6 Metabolism

7 NanoLuc® Technology Platform

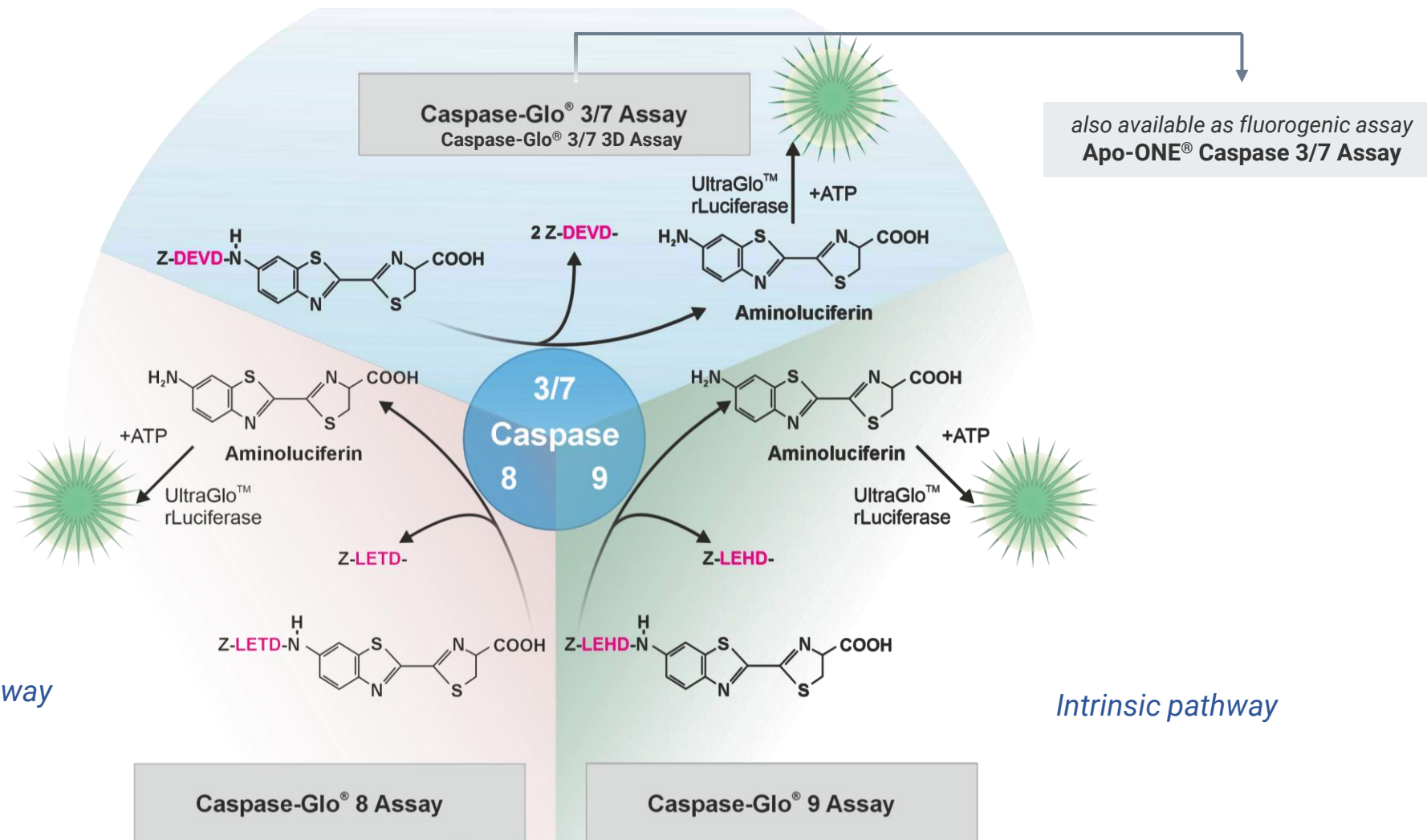
Caspase-Glo® Assays

**EFFECTOR
CASPASES**

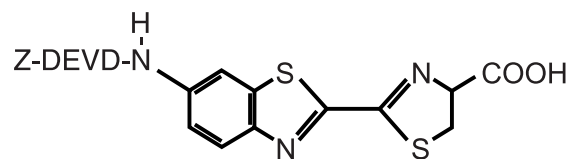
**INITIATOR
CASPASES**

Extrinsic pathway

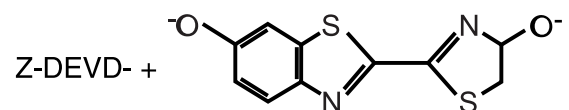
Intrinsic pathway



Multiplexing Caspase-Glo® 3/7 3D Assay in Spheroids



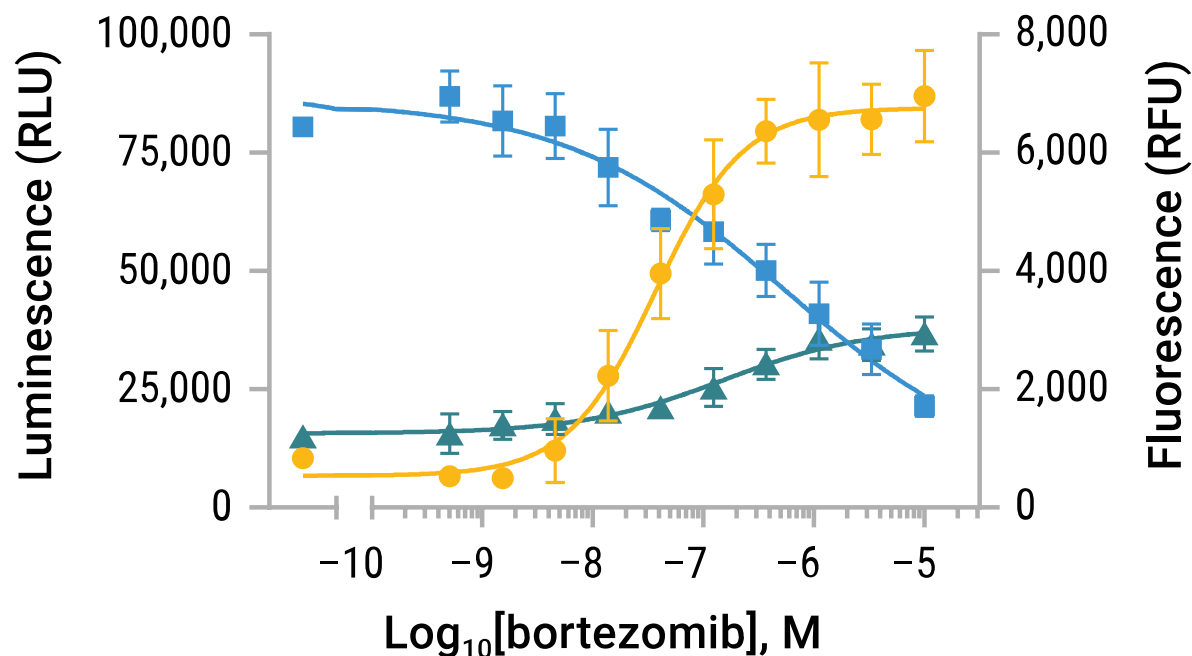
Caspase 3/7



Ultra-Glo™
rLuciferase

ATP, Mg²⁺, O₂

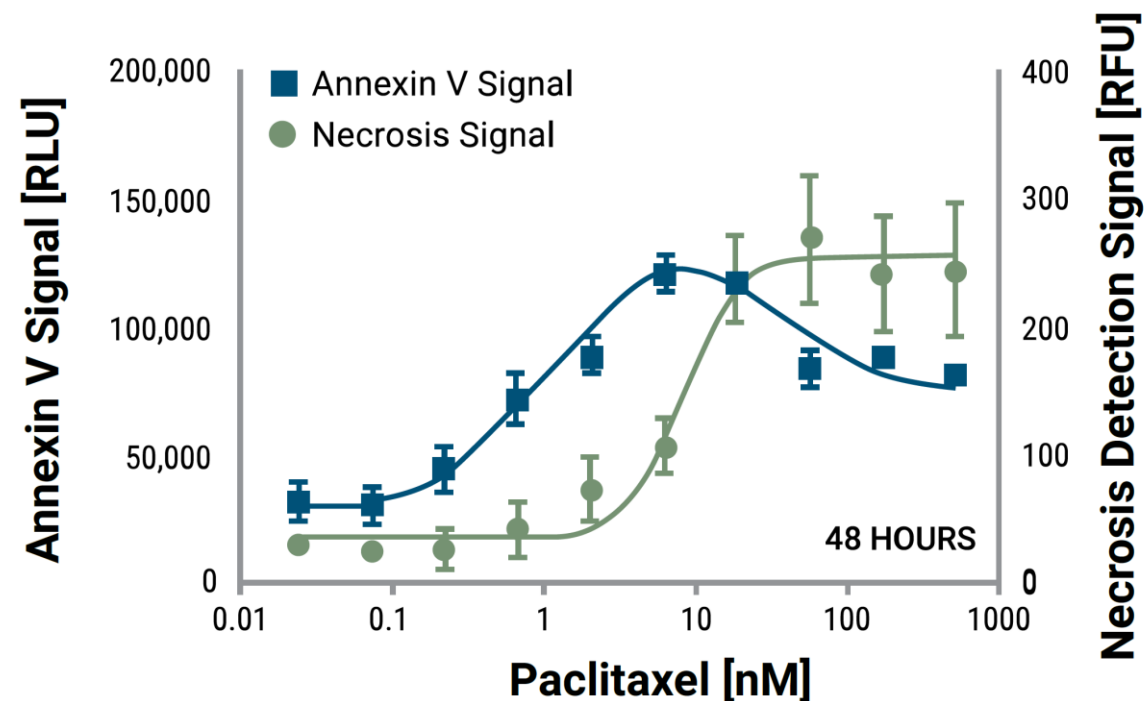
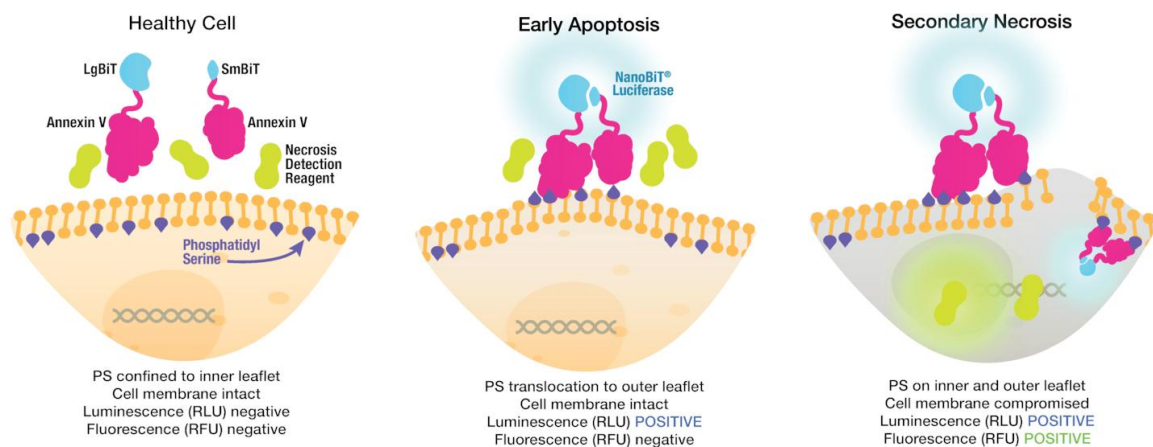
Glo



● Caspase-Glo® 3/7 3D ■ CellTiter-Fluor™ ▲ CellTox™ Green

A549 spheroids (average diameter of 450µm)

RealTime-Glo® Annexin V Apoptosis and Necrosis Assay



HepG2 spheroids were treated for 48 hours with different concentrations of paclitaxel

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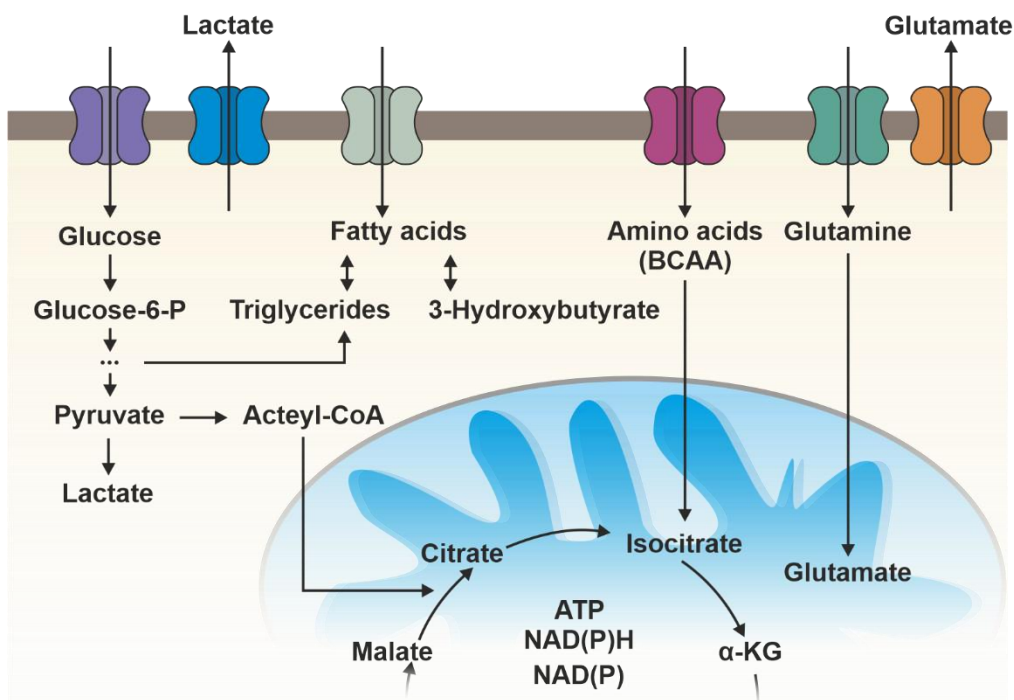
5 Apoptosis

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Promega Metabolism Assay Portfolio

Overview 2026



Glucose Metabolism

- Glucose-Glo™ Assay
- Glucose Uptake-Glo™ Assay
- Lactate-Glo™ Assay
- Glycogen-Glo™ Assay

Lipid Metabolism

- Glycerol-Glo™ Assay
- Triglyceride-Glo™ Assay
- Cholesterol/Cholesterol Ester-Glo™ Assay
- FAO-Glo™ Fatty Acid Oxidation Assay *
- BHB-Glo™ (Ketone Body) Assay

Amino Acid Metabolism

- Glutamate-Glo™ Assay
- Glutamine/Glutamate-Glo™ Assay
- BCAA-Glo™ Assay

TCA Cycle

- Malate-Glo™ Assay
- Pyruvate-Glo™ Assay

Metabolic Co-factors

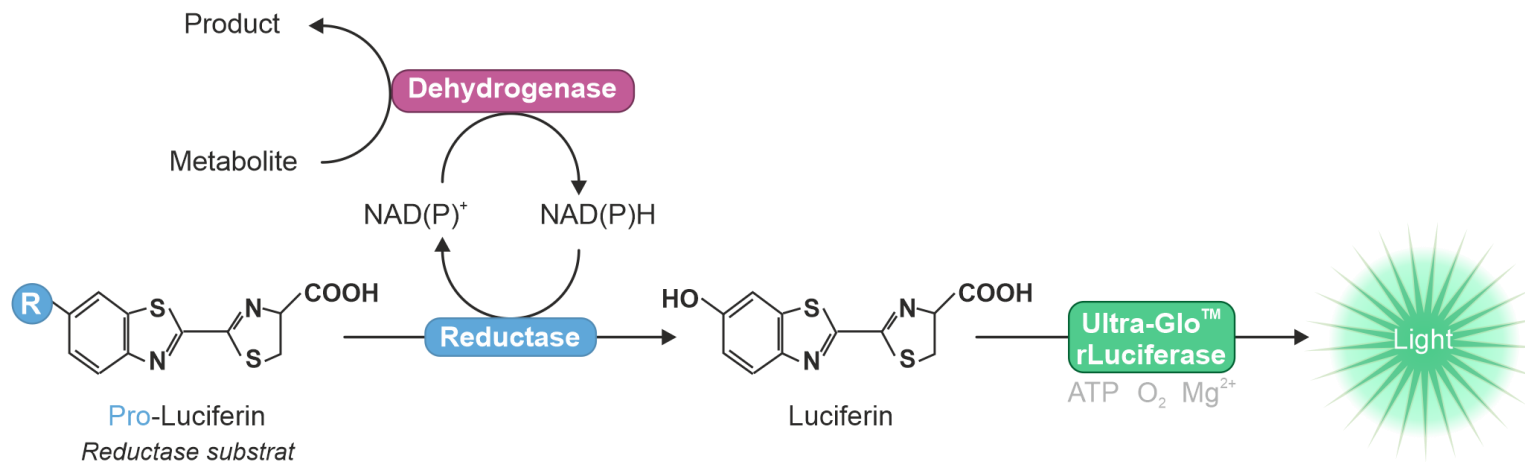
- NAD/NADH-Glo™ Assay
- NADP/NADPH-Glo™ Assay
- CellTiter-Glo® Assay (ATP)

DIY

- Dehydrogenase-Glo™ Detection System
- Metabolite-Glo™ Detection System

* available as Early Access Product

Promega Metabolism Assay Portfolio



Ultra-Glo™ rLuciferase

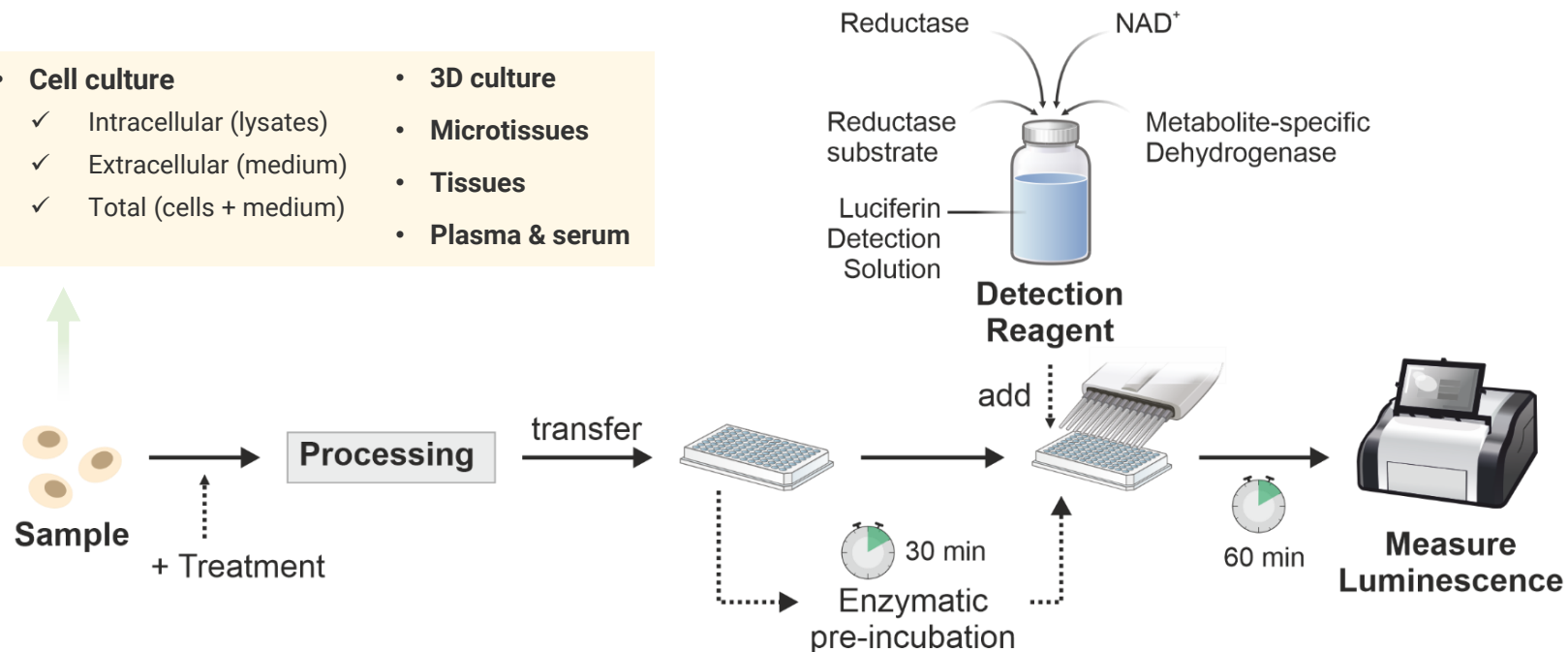
- Greater thermal stability
- Greater pH tolerance
- Greater detergent/reducing agent tolerance
- Reduced compound interference

- Rely on core reaction of the NAD(P)H-Glo™ Detection System
- Use a NAD(P)-dependent **dehydrogenase** that **oxidizes metabolite** of interest

- **Light** output is **dependent on [metabolite]**

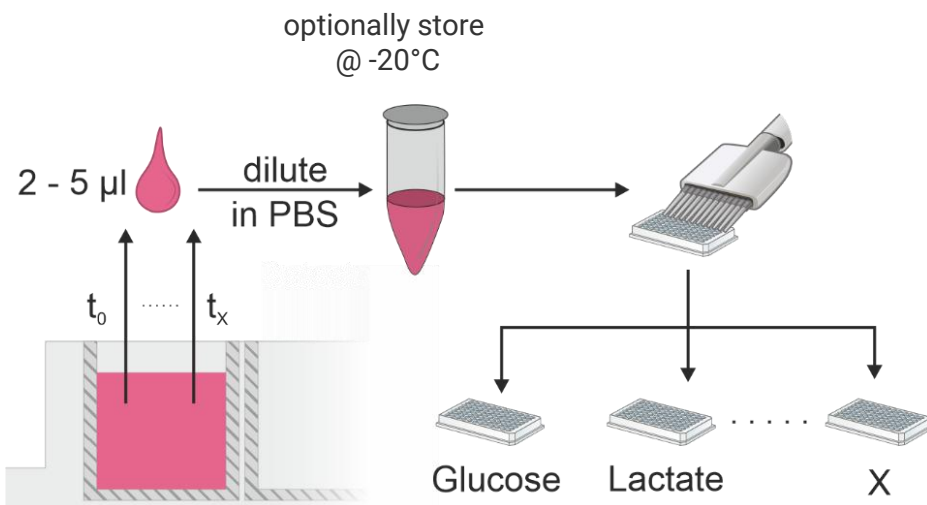
Sample Compatibility & Workflow Simplicity

- **Cell culture**
 - ✓ Intracellular (lysates)
 - ✓ Extracellular (medium)
 - ✓ Total (cells + medium)
- **3D culture**
 - **Microtissues**
 - **Tissues**
 - **Plasma & serum**



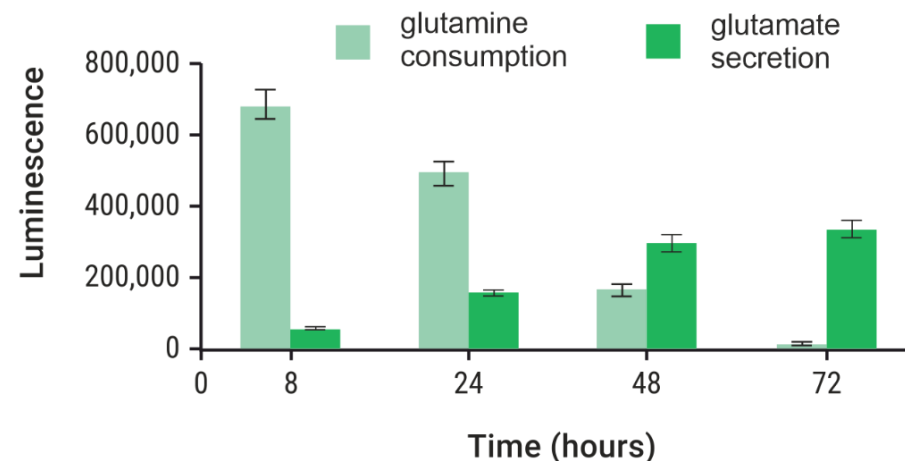
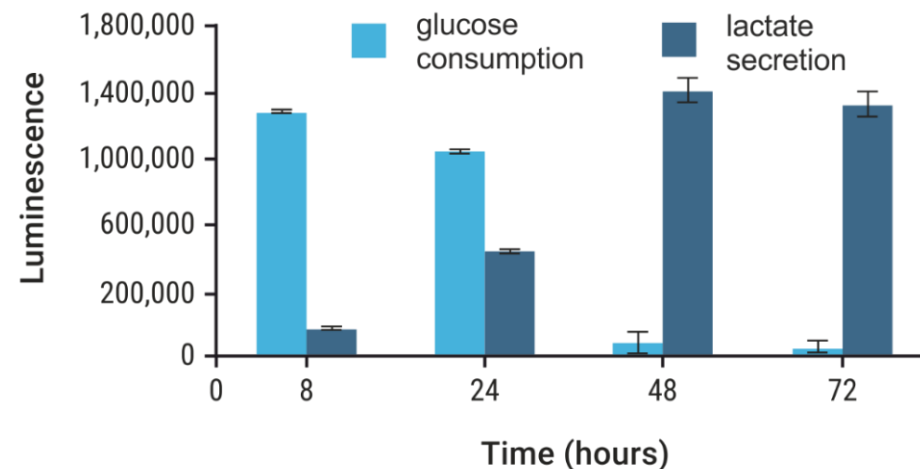
- Easy workflow, applicable to various sample types
- Technical Manual contains recommendations for processing of different sample types
- Can be combined with other energy metabolism assays due to compatible processing

Measuring Metabolite Levels in Supernatant



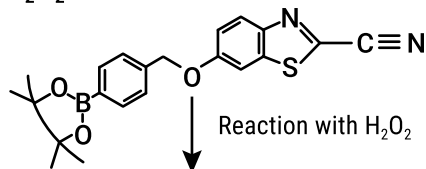
- Easy, convenient, compatible and homogenous workflow
- Multiplexing compatible, e.g. with cell viability assays
- Maximize data output by allowing kinetic multiparameter analysis from a single sample

A549; 15,000 cells/well; 1:40 dilutions of 2.5 μl aliquots

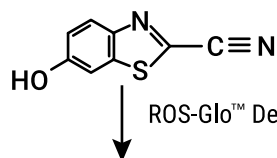


Oxidative Stress Assay: ROS-Glo™ H2O2 Assay

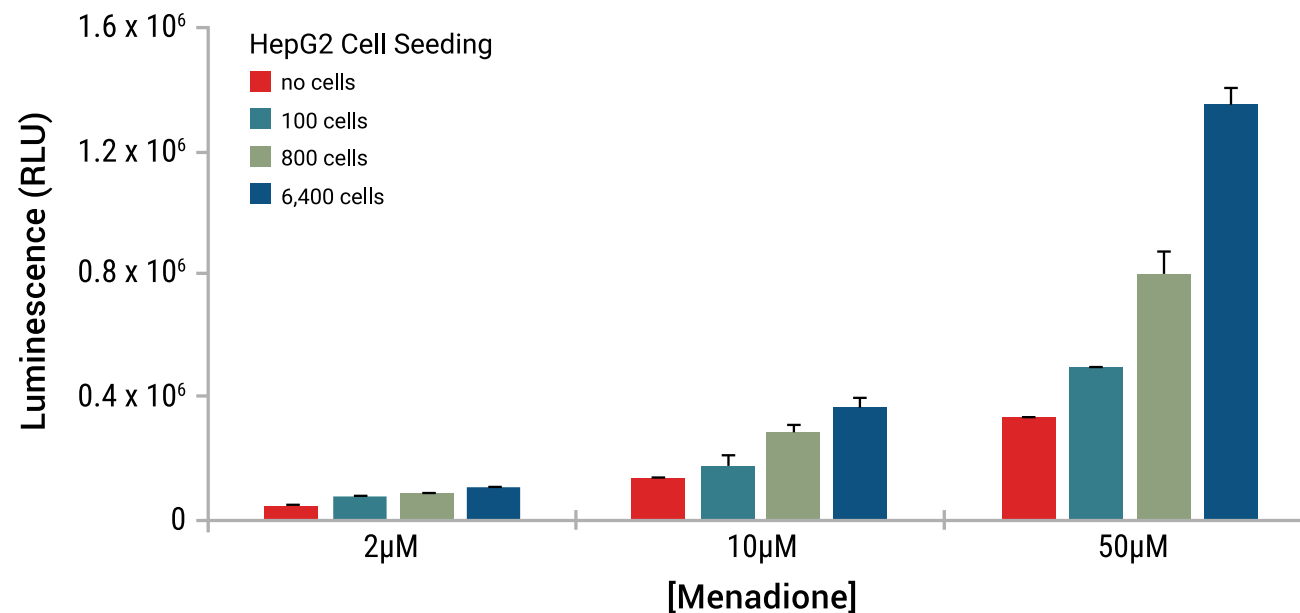
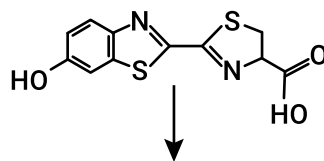
H₂O₂ Substrate



Luciferin Precursor

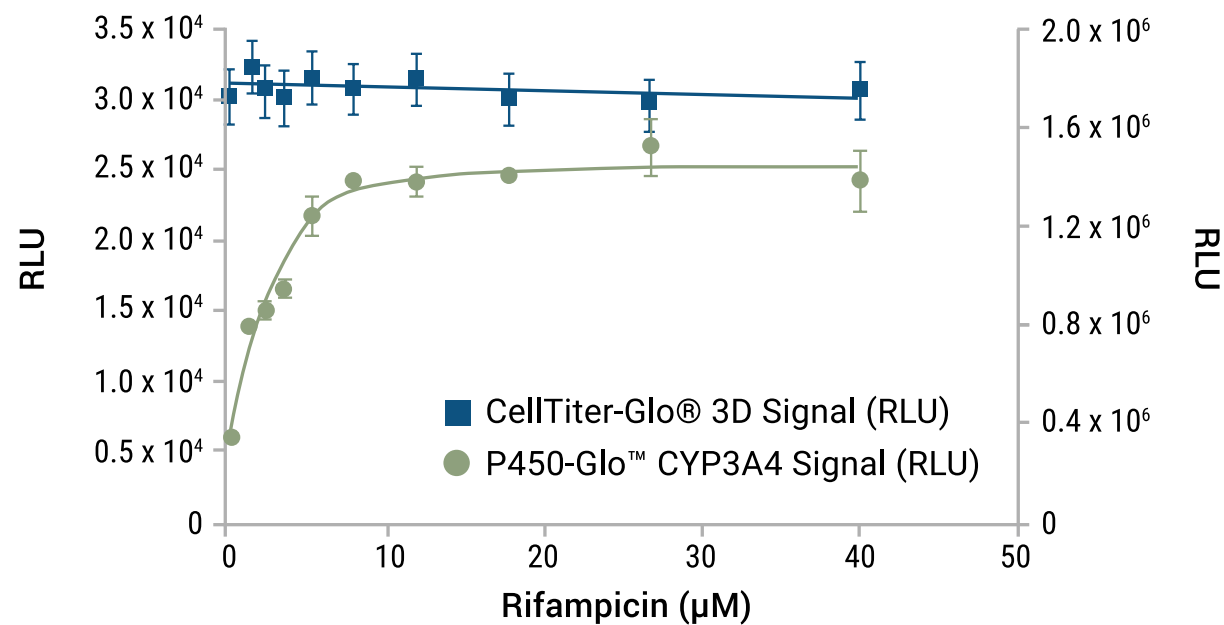
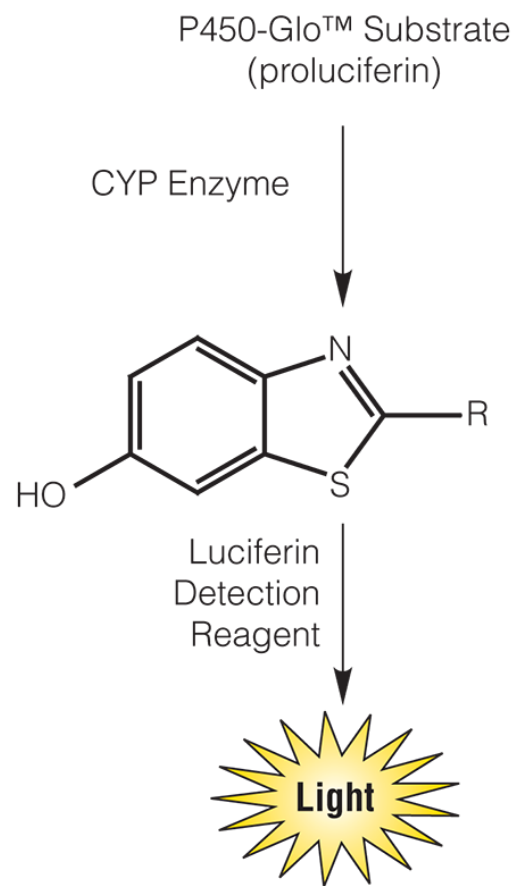


Luciferin



HepG2 cells were grown as spheroids in ultra-low attachment plates (Corning) for 4 days and treated with menadione.

Drug Metabolism Assay: P450-Glo™ CYP3A4 Assay



Monitoring human liver microtissue Cytochrome P450 3A4 activity

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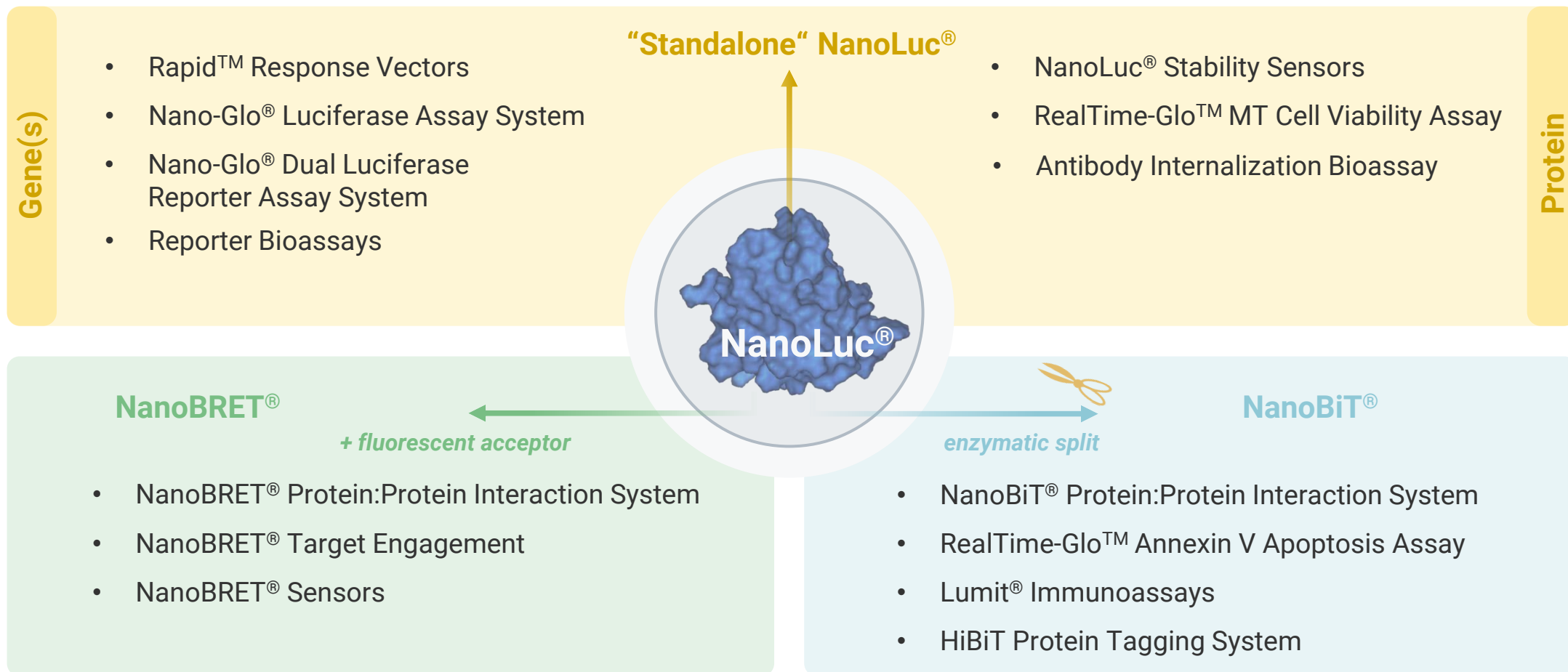
4 Proliferation

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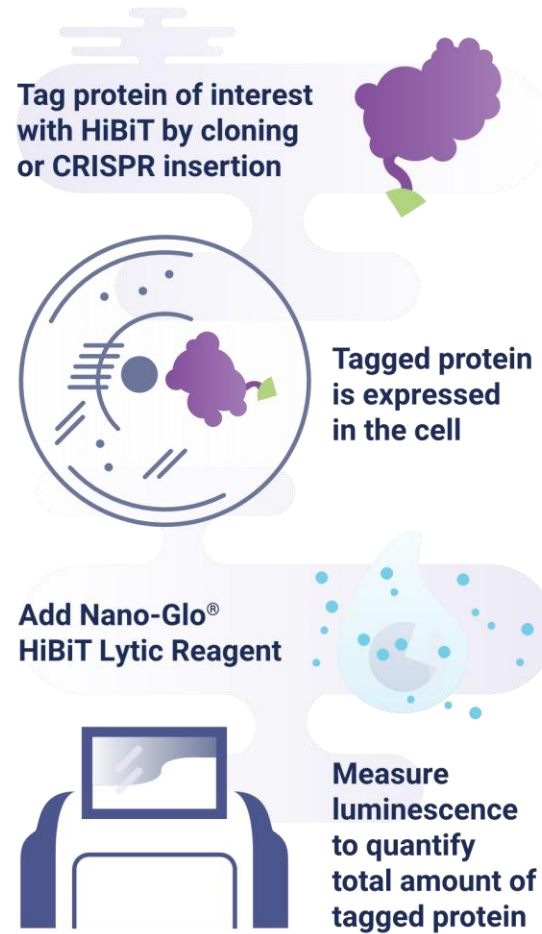
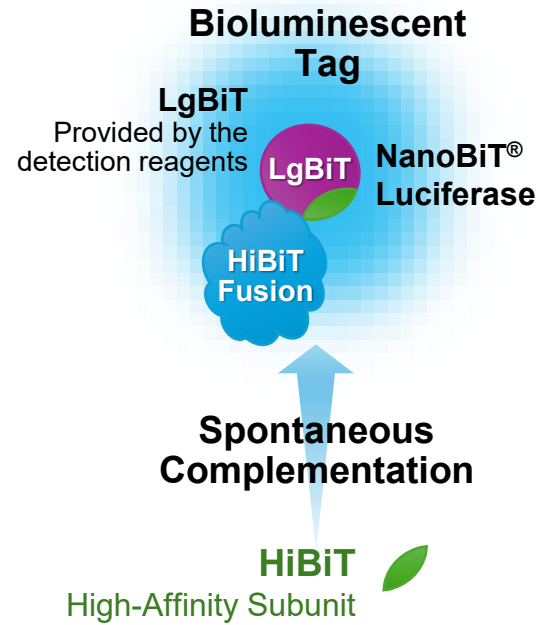
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7 NanoLuc® Technology Platform

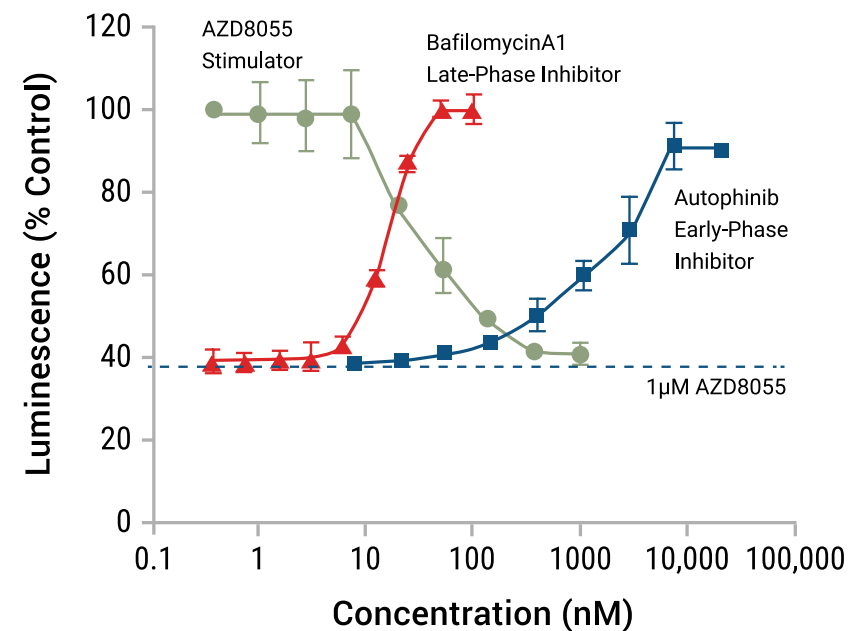
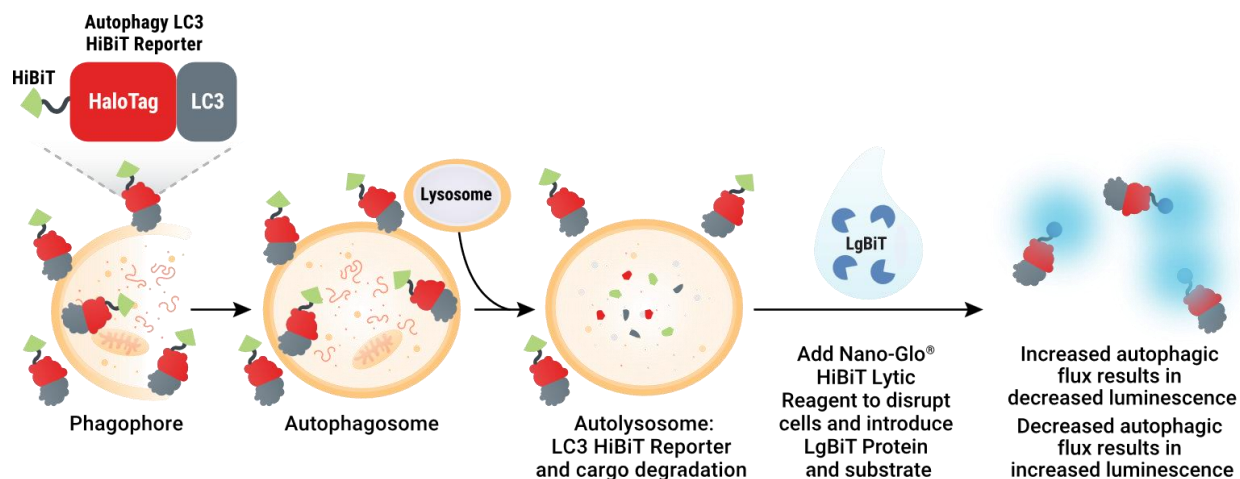
NanoLuc® Luciferase Technology Platform



HiBiT Protein Tagging System

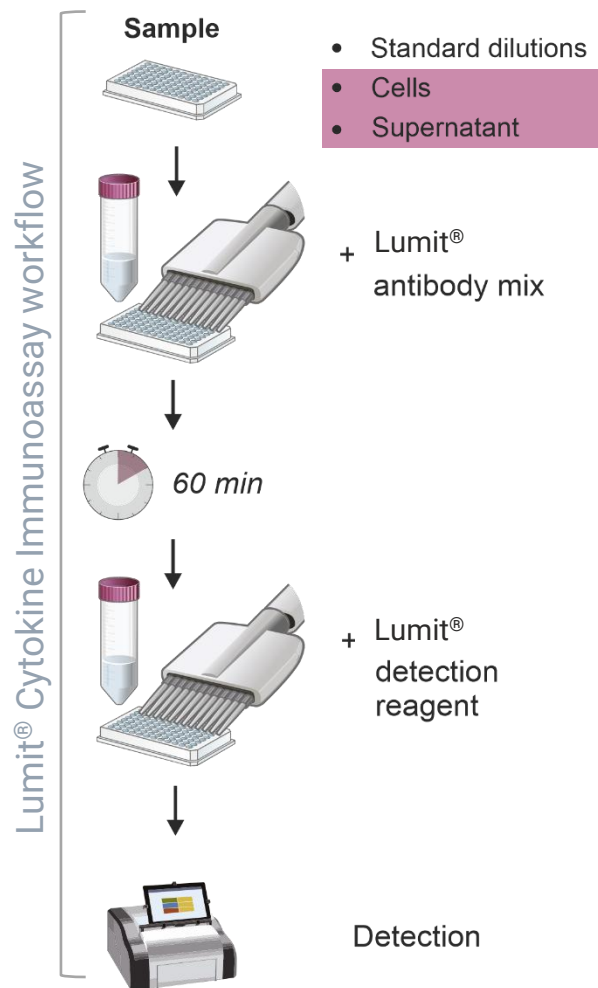


Autophagy LC3 HiBiT Reporter Assay System

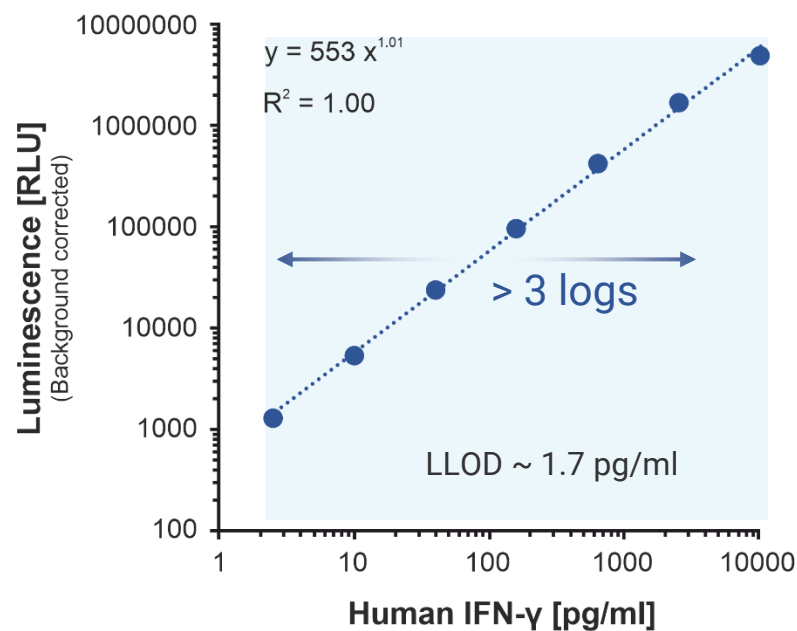


HEK293 LC3 HiBiT reporter spheroids (~350 μ m; 500 cells/well) were grown for 4 days in a 96-well ultra-low attachment plate, then treated with autophagy modulators for 6 h

Lumit® Cytokine Immunoassays



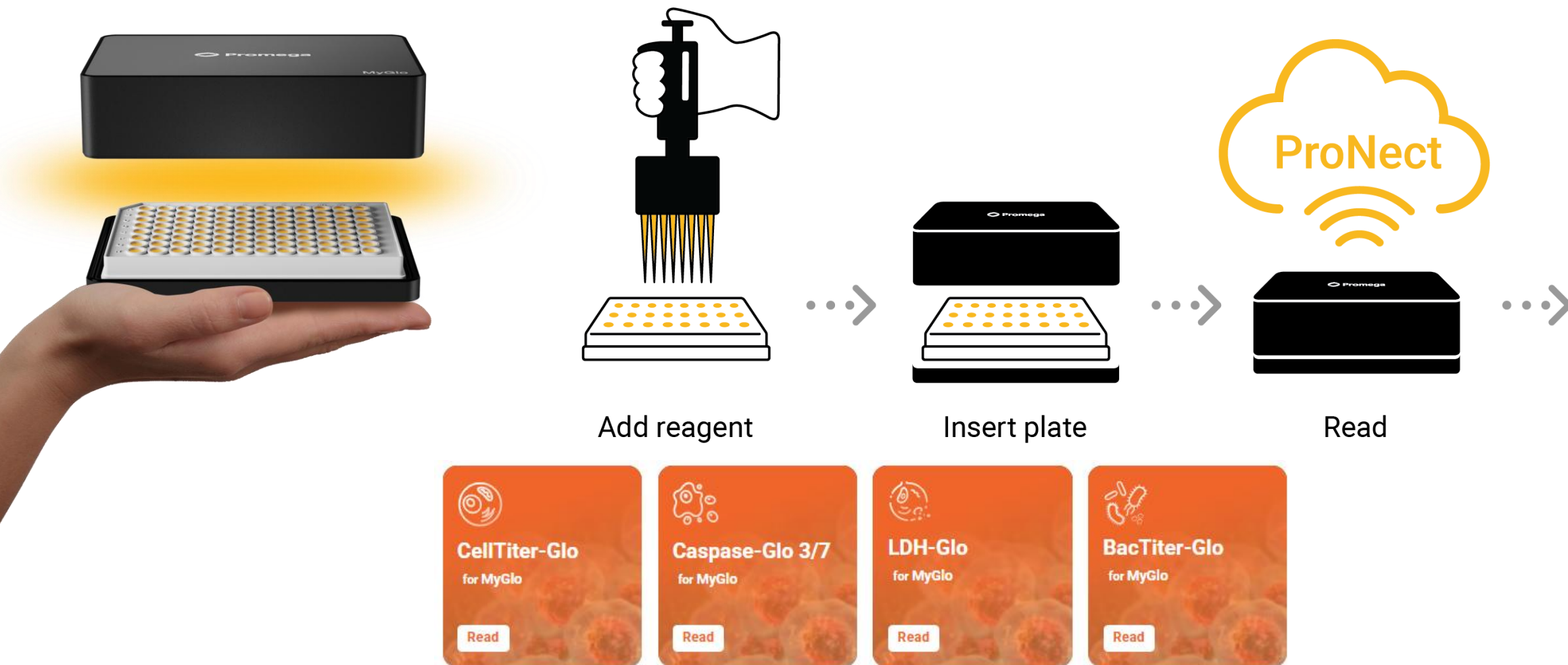
Lumit® (human) IFN-γ Immunoassay



Assay	Dynamic range	LOD
Lumit® IL-2 (Human)	28.2 – 25000 pg/ml	11.2 pg/ml
Lumit® IL-4 (Human)	18.2 – 25000 pg/ml	6.7 pg/ml
Lumit® IL-6 (Human)	18.2 – 25000 pg/ml	7.5 pg/ml
Lumit® IL-10 (Human)	18.2 – 25000 pg/ml	7.4 pg/ml
Lumit® IFN-γ (Human)	7.2 – 10000 pg/ml	1.7 pg/ml
Lumit® TNF-α (Human)	18.2 – 25000 pg/ml	2.9 pg/ml
Lumit® IL-12 p70 (Human)	18.2 – 25000 pg/ml	10.4 pg/ml
Lumit® IL-1β (Human)	22 – 40000 pg/ml	10 pg/ml
Lumit® IL-1β (Mouse)	11 – 20000 pg/ml	8 pg/ml
Lumit® HMGB1 Human/Mouse Immunoassay	4 – 1000 ng/ml (hu) 3 – 2187 ng/ml (ms)	1 ng/ml 3 ng/ml
Lumit® Active IL-18 (Human)	11 – 20000 pg/ml	2 pg/ml
Lumit® IL-8 (Human)	7.29 – 10000 pg/ml	1.7 pg/ml
Lumit® IL-17A (Human)	18.2 – 25000 pg/ml	3 pg/ml
Lumit® IFN-β (Human)	18.2 – 25000 pg/ml	5.3 pg/ml
Lumit® VEGF-A (Human)	17.3 – 7500 pg/ml	4.8 pg/ml

- Good sensitivity and broad dynamic range (~ 3 logs)
- No need for sample dilution
- Flexible detection protocols

MyGlo® Reagent Reader - Portable 96-Well Luminescence Plate Reader



GloMax® Systems

Plate reader



GloMax® Navigator *

- 96-well plates
- Luminescence



GloMax® Explorer *

- 6- to 384-well plates (+/- lid)
- Luminescence
- Fluorescence
- Vis Absorbance
- Heating / Shaking



GloMax® Discover *

- 6- to 384-well plates (+/- lid)
- Luminescence
- Fluorescence
- UV / Vis Absorbance
- Heating / Shaking
- BRET / FRET

Live-Cell Imager

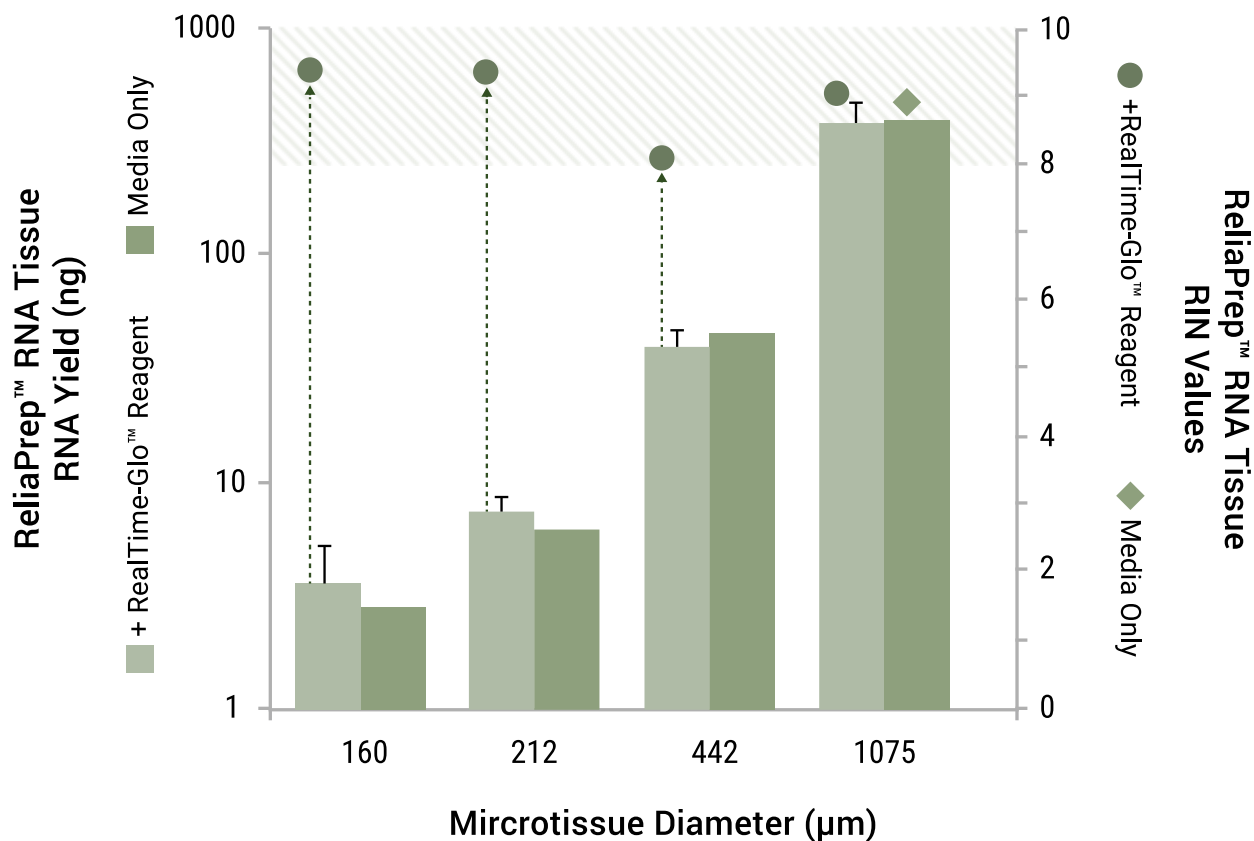


GloMax® Galaxy **

- (chamber-)slides, 35 mm dish, 6- to 96-well plates
- Luminescence
- Fluorescence
- Brightfield
- BRET
- 10.3X Magnification

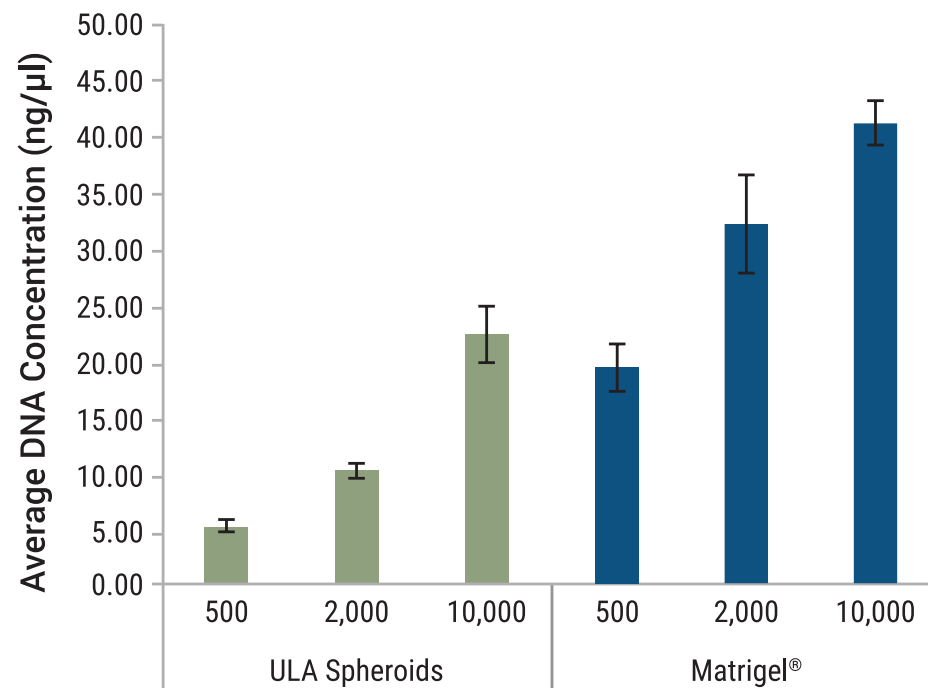
* Optional: two channel injector system ** Optional: stagetop incubator

ReliaPrep™ RNA Miniprep Systems



Extraction of RNA from HEK293 Microtissue Spheroids. The ReliaPrep™ RNA Miniprep Systems synergize with non-lytic cellular assays like the RealTime-Glo™ MT Cell Viability Assay.

Maxwell® RSC Cultured Cells DNA Kit

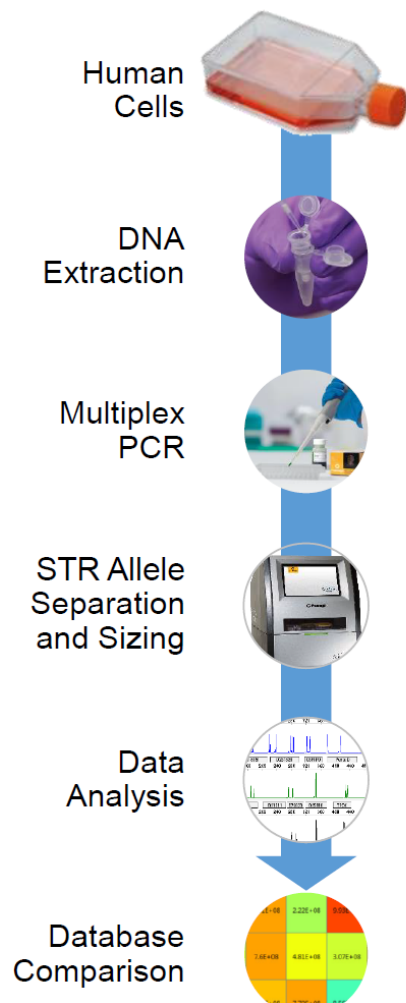


3D Culture Type and Number of Seeded Cells

DNA was purified from spheroids grown by two 3D culture methods (500, 2,000 and 10,000 cells) using the Maxwell® RSC Cultured Cells DNA Kit and quantified with the QuantiFluor® ONE dsDNA System.

STR Profiling Workflow

STR Profiling Workflow



All or part of this workflow offered by CLA service providers which could include Genetic Core facilities.

[Learn More](#)



Product	Cat. #	Size
GenePrint® 10 System	B9510	50 rxns
GenePrint® 24 System	B1870	100 rxns

Both Systems are compatible with the Spectrum Compact CE System and with the Applied Biosystems® Genetic Analyzers.

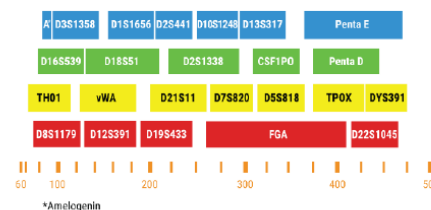
Panels and bins text files are required for automatic assignment of genotypes using the GeneMapper® and GeneMarker® software and are available for download. Please contact Promega Technical Services if you need assistance.



Bring *GenePrint®* 10 or 24 System analysis to your own lab with the [Spectrum Compact CE Instrument](#)

Recommended for patient-derived cell lines and meets the ASN-0002-2011 Guidelines

The *GenePrint®* 24 System allows co-amplification and five-color detection of 24 loci



Generally sufficient for established cell lines

GenePrint® 10 provides co-amplification and four-color detection of 10 loci



Thank You!



Děkuju

For additional questions please contact:
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