Promega

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

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EASTPORT LIFESCIENCE

Our Mission

Provide innovative biological reagents and integrated systems used in research and applied technology worldwide.

Supporting Science Around the World

100+
Countries

50+
Distributors

- Headquarters
- R&D and Manufacturing
- Branch Office

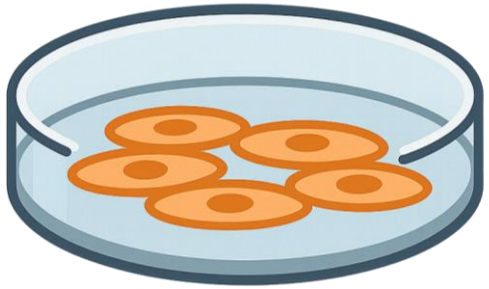
Product Portfolio

DNA & RNA Analysis	Cellular Analysis	Protein Analysis	Genetic Identity	Molecular Diagnostic	Drug Development
• DNA and RNA Purification • DNA Amplification PCR and qPCR • Reverse Transcription and RNA protection • Sequencing Sample Preparation • Cloning, Enzymes and DNA Markers • Transfection and Epigenetics	• Cell Health (viability, cytotoxicity, apoptosis) • Cellular Metabolism • Cell Signaling • Reporter Assays • Imaging	• Mass Spectrometry • Immunoassays • Protein Quantification • Protein Expression • Protein Purification • Protein Interaction	• Forensic and Paternity Testing • STR Typing	• cGMP Manufacturing • Gene Analysis and Mutation Determination	• Biologics • Small-Molecule Drug Discovery
Instrumentation					
• Instruments for DNA and RNA Extraction and Quantification	• Luminometer, Fluorometer and Bioluminescence Imager		• Capillary Electrophoresis Systems		

2D Cell Culture – The Foundation of In Vitro Biology

Advantages

- Simple and reproducible
- Cost-effective, scalable
- Ideal for mechanistic and screening studies



AI generated image

Disadvantages

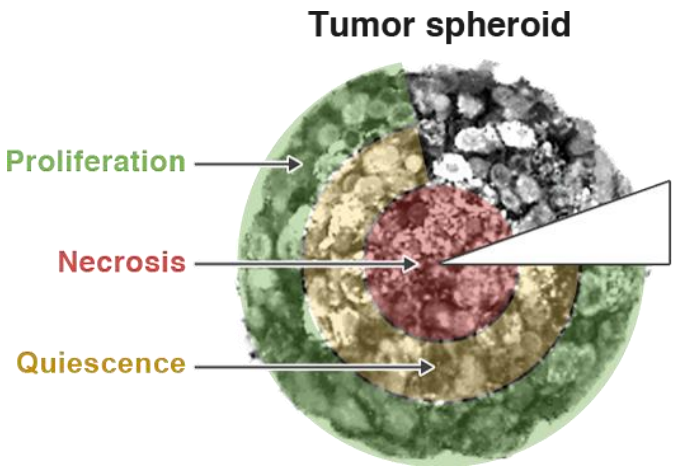
- Flat, non-physiological growth
- Uniform nutrients and oxygen
- Limited cell–cell and ECM interactions

The nature and importance of metabolic restriction in cancer has often been masked owing to the use of *tissue culture conditions in which both oxygen and nutrients are always in excess*.

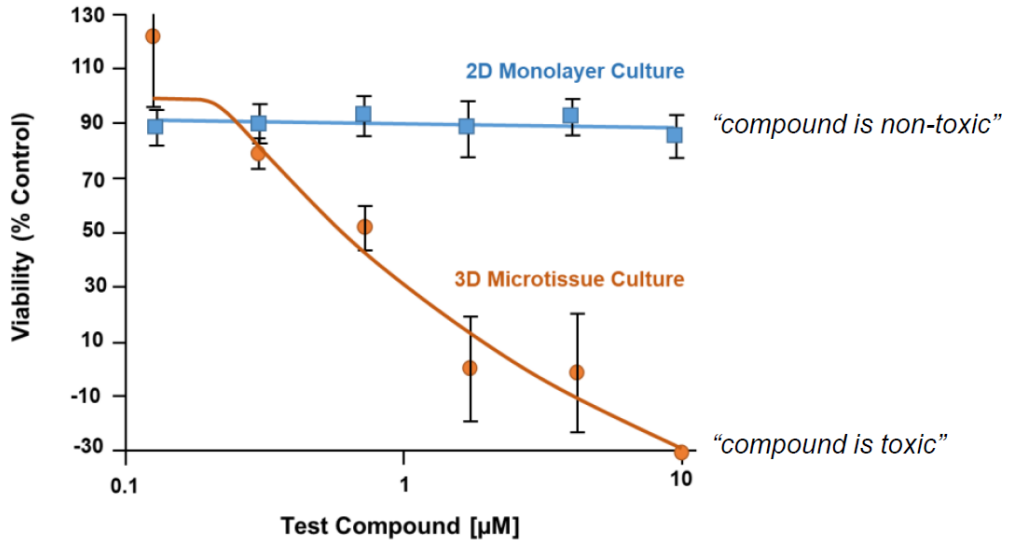
Cairns, R.A., Harris, I.S. and Mak, T., (2011) Regulation of Cancer Cell Metabolism. *Nature Reviews: Cancer* **11**, 85-95.

3D Cell Culture - Bridging the Gap to Physiological Relevance

- Mimics tissue/tumor more closely:
  - Center is more hypoxic
  - Nutrients more limiting
- Allows more natural 3D cell-to-cell contacts
- Allows more natural interactions with matrix (and matrix creation)



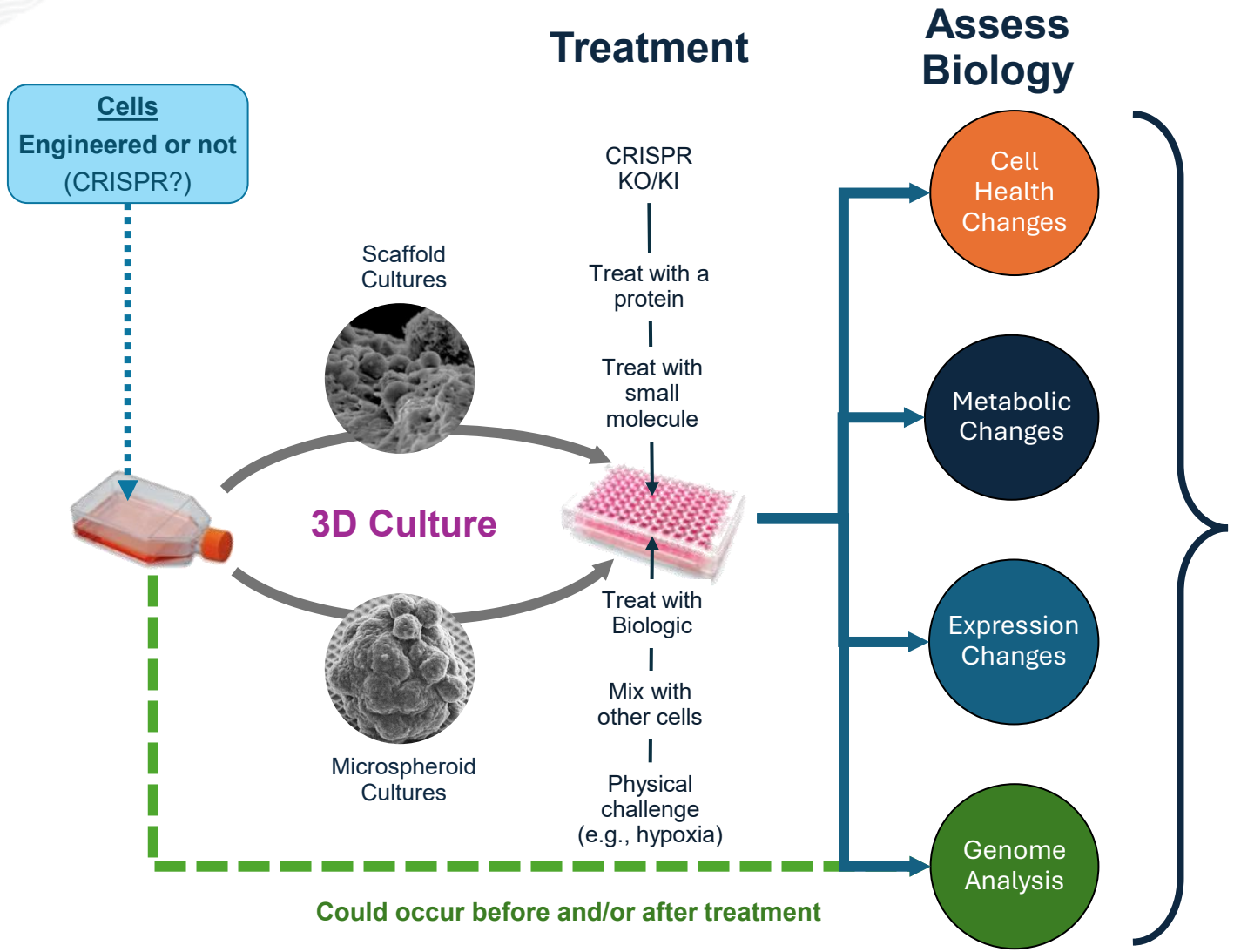
3D Culture can Reveal Dramatic Differences in Cellular Responses



How the 3D Environment Is Generated

Types	Key Principle	Examples
Scaffold-Free	Cells self-assemble into 3D aggregates without external support	Spheroids, organoids, cell sheets
Scaffold-Based	External materials mimic ECM and provide structural/mechanical support	Hydrogels (collagen, alginate, Matrigel), polymers (PLGA, PEG), decellularized ECM
Bioprinted	Cells and biomaterials are deposited layer-by-layer to build 3D structures	Cell-laden hydrogels, printed tissue strands, hybrid constructs

Goal is to measure biology following treatment…



Do you have the right tools to measure the biology?

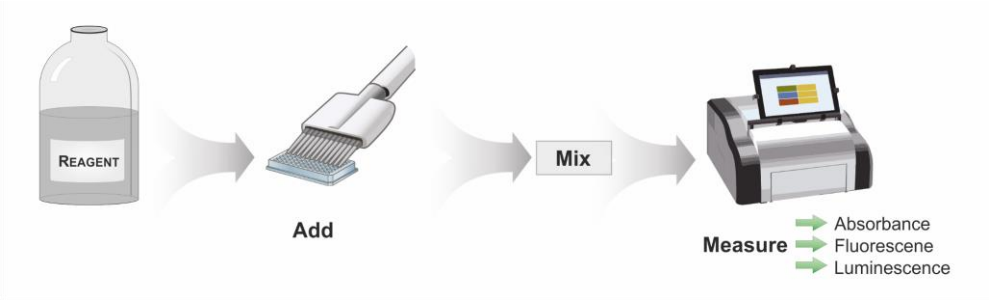
Most available cellular assays were developed with 2D monolayer cultures

Easy to lyse and release cellular contents

3D microtissue cultures challenges:

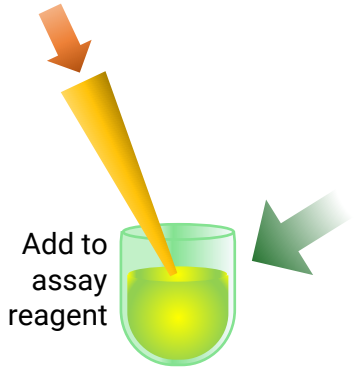
- Harder to penetrate
- Cells set down their own matrix
- Conditions for rapid lysis may destroy the enzyme you wish to measure

Promega's Cell-based Assay Portfolio



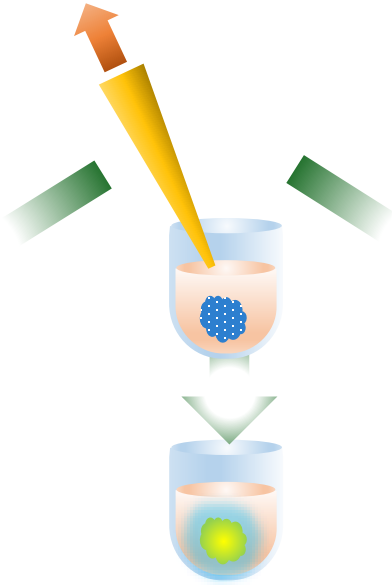
Benefits

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



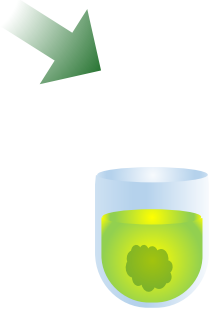
Media Sampling
Non-Lytic Assays

LDH-Glo™ Cytotoxicity Assay



Live Cell Assays
Non-Lytic Assays

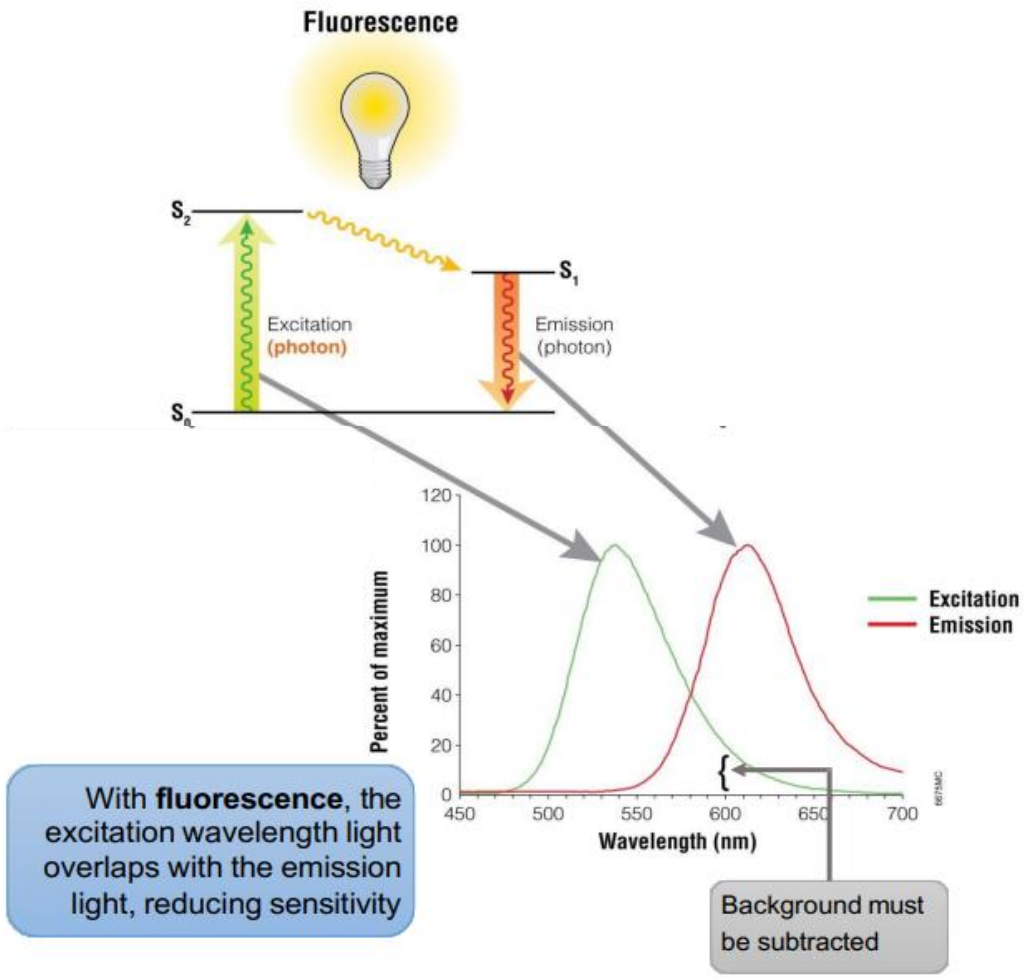
RealTime-Glo™ MT Cell Viability Assay

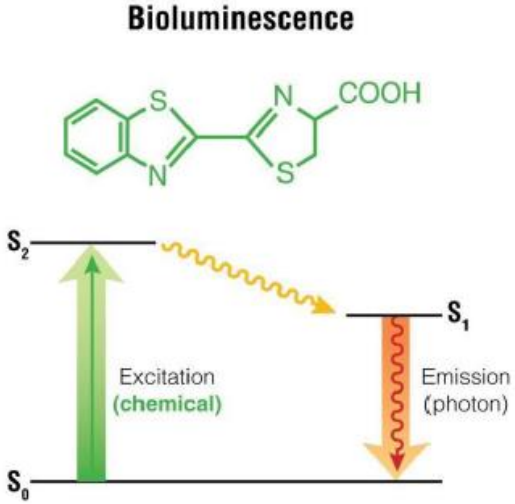


End-Point
Lytic Assays

CellTiter-Glo® 3D Cell Viability Assay

Why Bioluminescence?

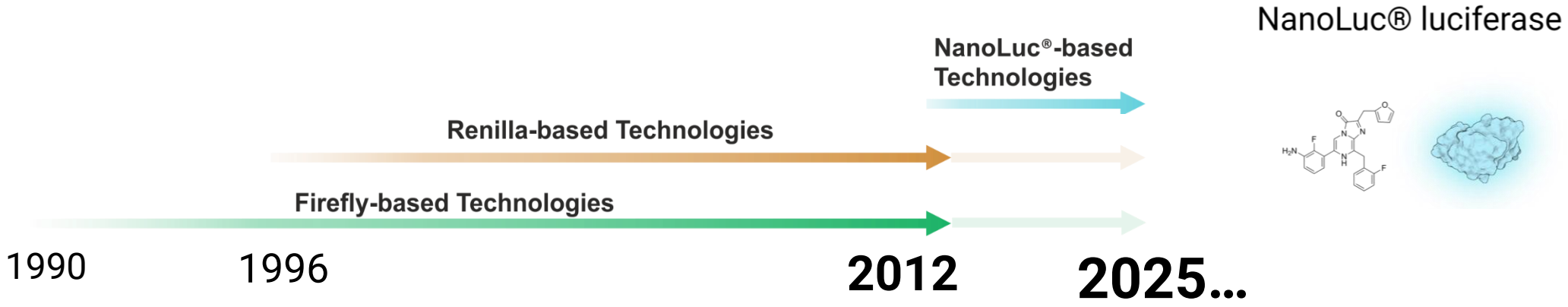




With **luminescence**, the energy input is chemical in nature so background is minimal and, thus, more sensitive.

The added sensitivity gained from the minimal background makes bioluminescence the ideal choice for plate-based assays.

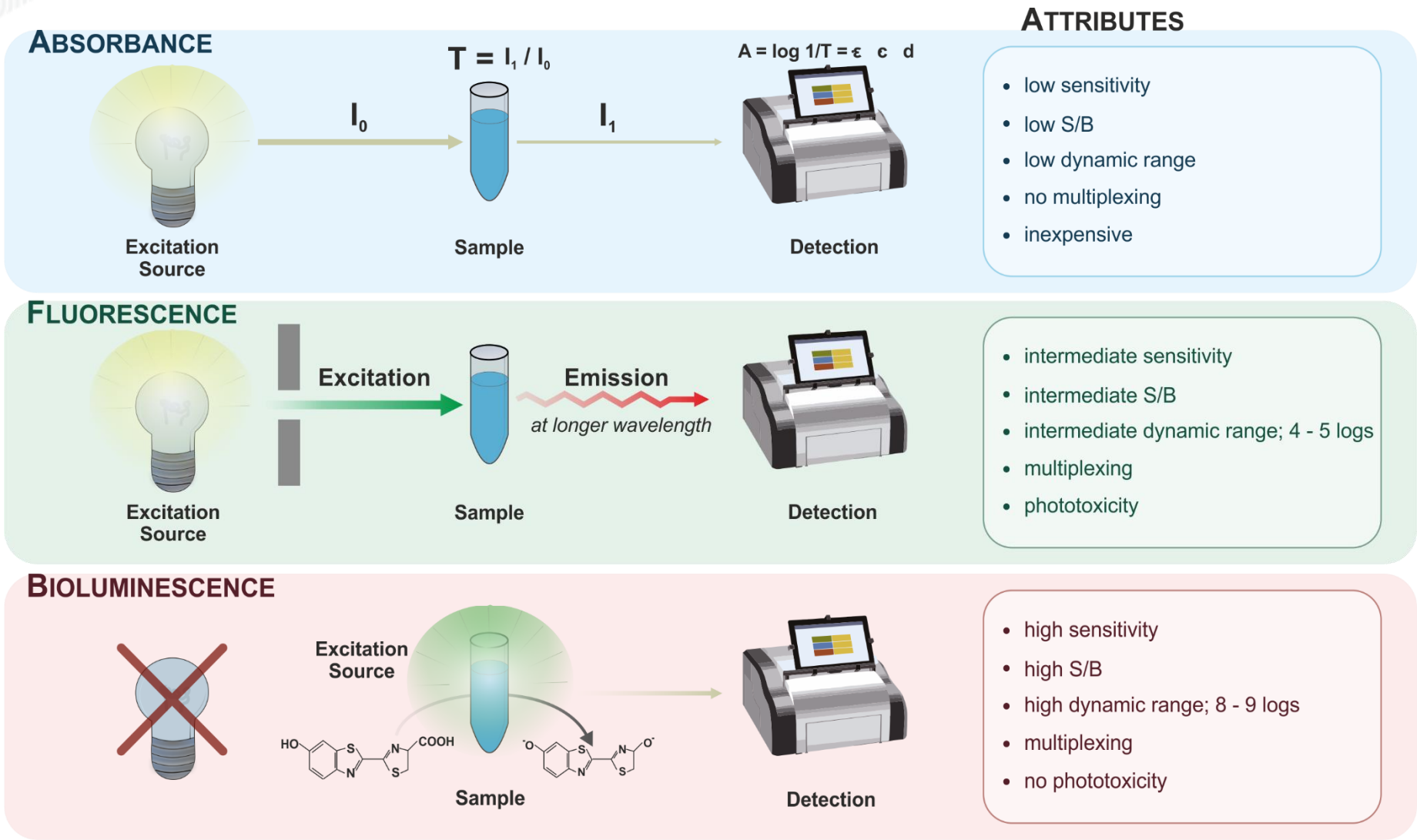
Decades of Experience with Bioluminescence



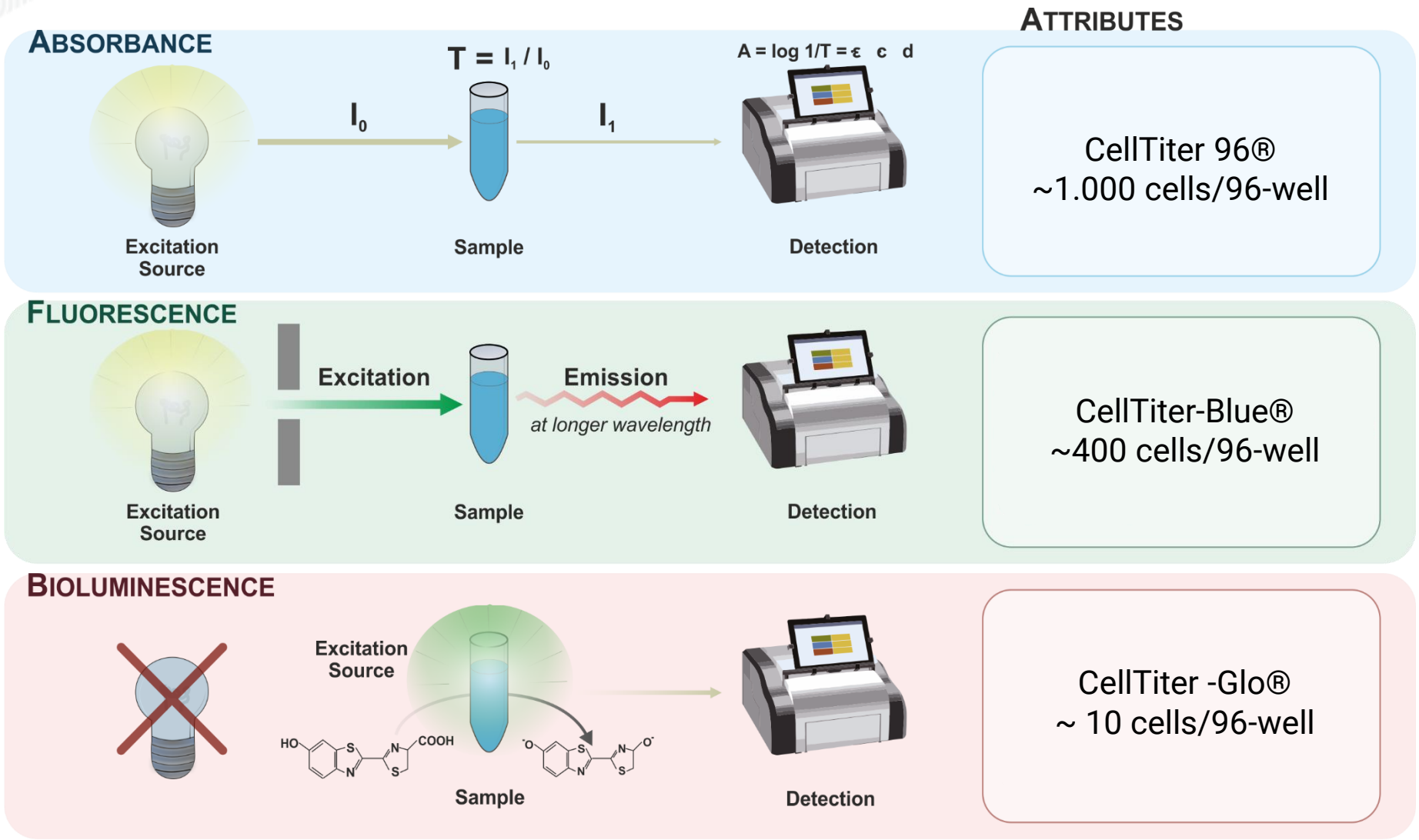
- Reporter Gene Assays
- GloSensor™ (cAMP, Protease Assays)
- GloResponse™ (Signaling Pathways)
- Rapid Response™ (Signaling Pathways)
- Cell-Health Assays
- Bioassays (ADCC, PDL1..)

- NanoBRET™ Target Engagement
- NanoBRET™ Protein:Protein Interaction
- NanoBiT® Protein:Protein Interaction
- HiBiT Protein Tagging System
- Lumit™ Immunoassays
- …

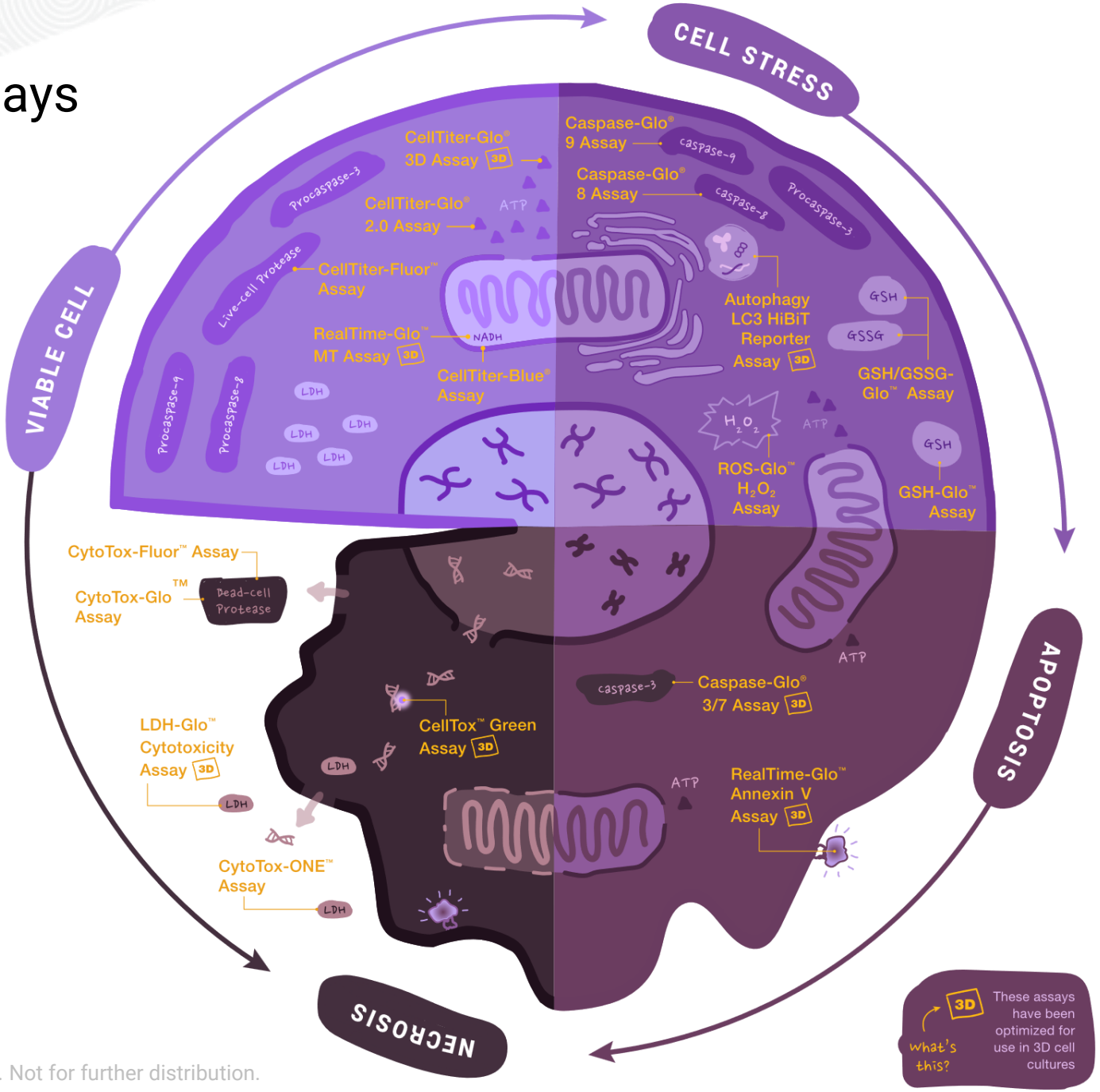
Assays Detection Systems



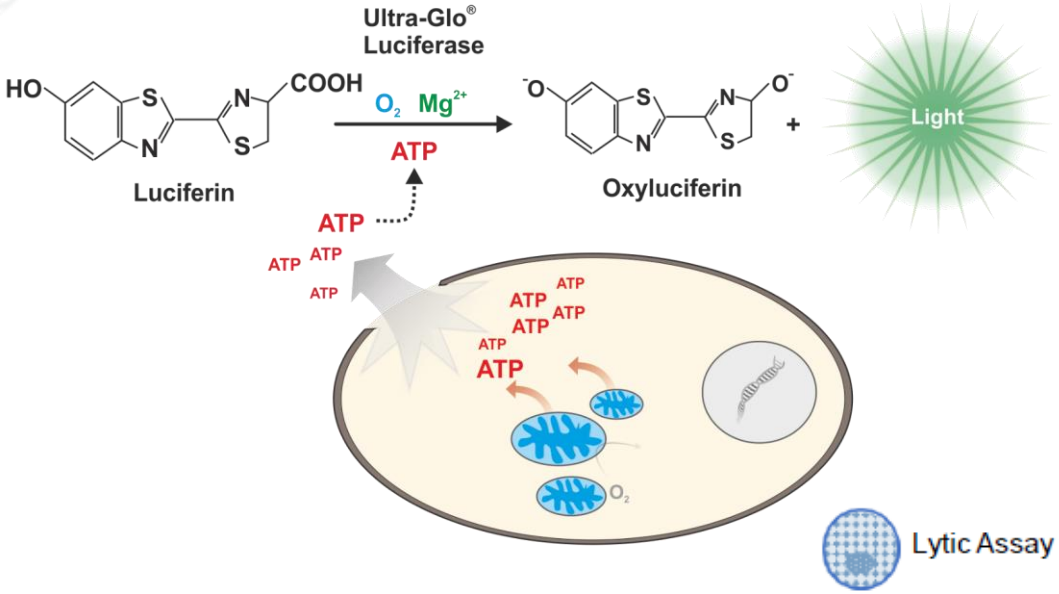
Assays Detection Systems

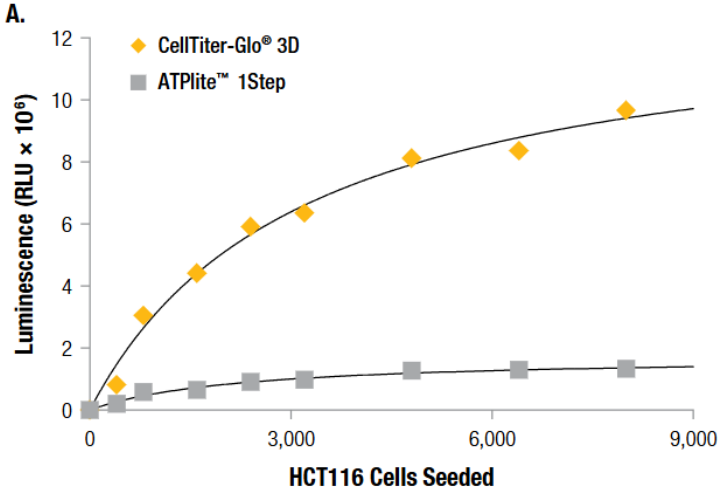


Cell Health Assays



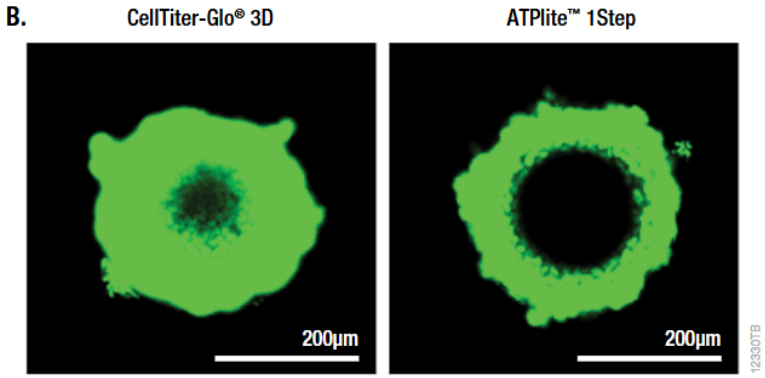
CellTiter-Glo® 3D Cell Viability Assay



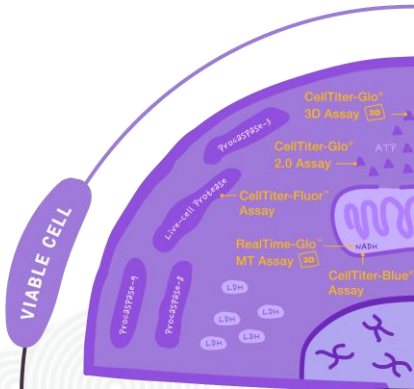


HCT116 colon cancer spheroids

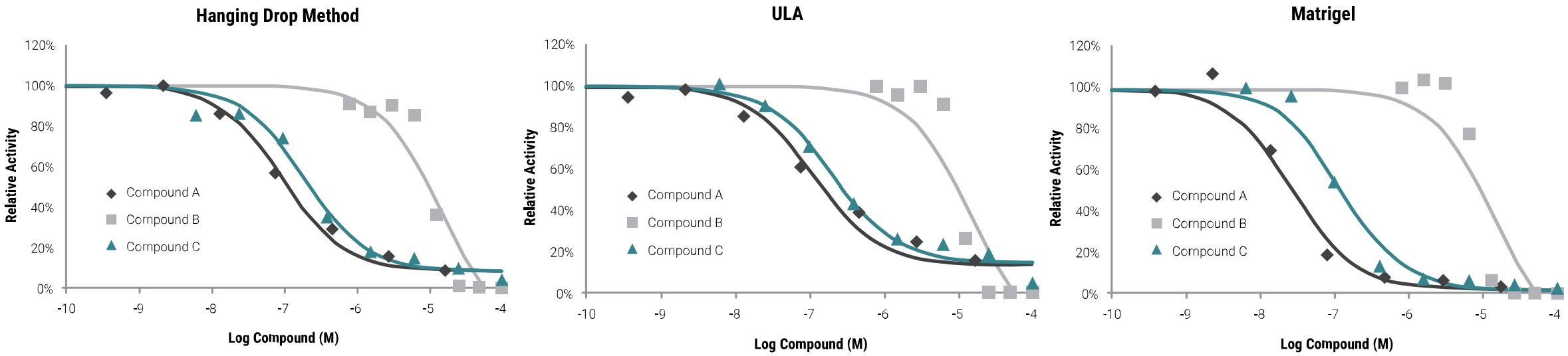
Improved 3D Microtissue Penetration, More Accurate Viability Data



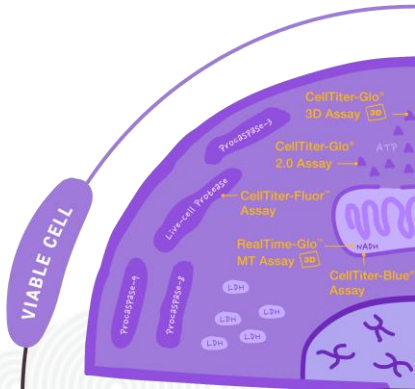
spheroid hydrogel organoid microfluidic



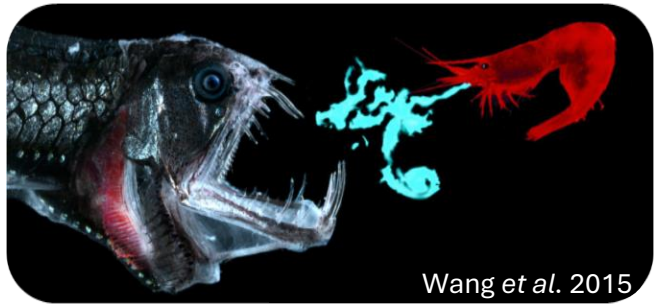
CellTiter-Glo® 3D Cell Viability Assay - Compatible with a Variety of 3D Culture Methods



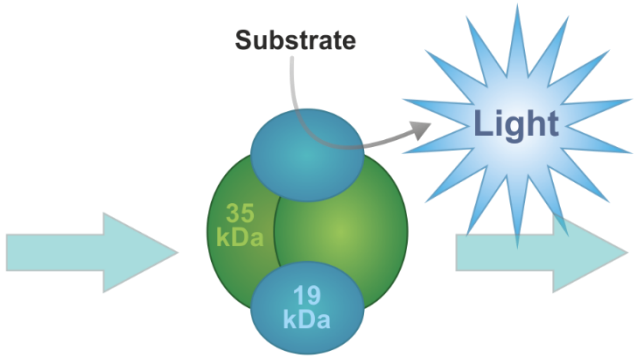
HCT116 colon cancer cells were seeded as follows: 400 cells in hanging-drop; 1,000 cells in ULA or Matrigel®. Microtissues were grown for 4 days, treated with compounds for 48 hours, and then assayed with the CellTiter-Glo® 3D Reagent. Luminescence was recorded at 30 minutes.



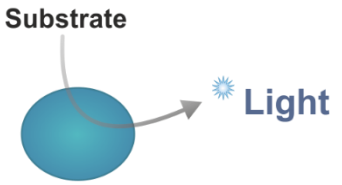
NanoLuc® Luciferase: A Bright and Small Reporter



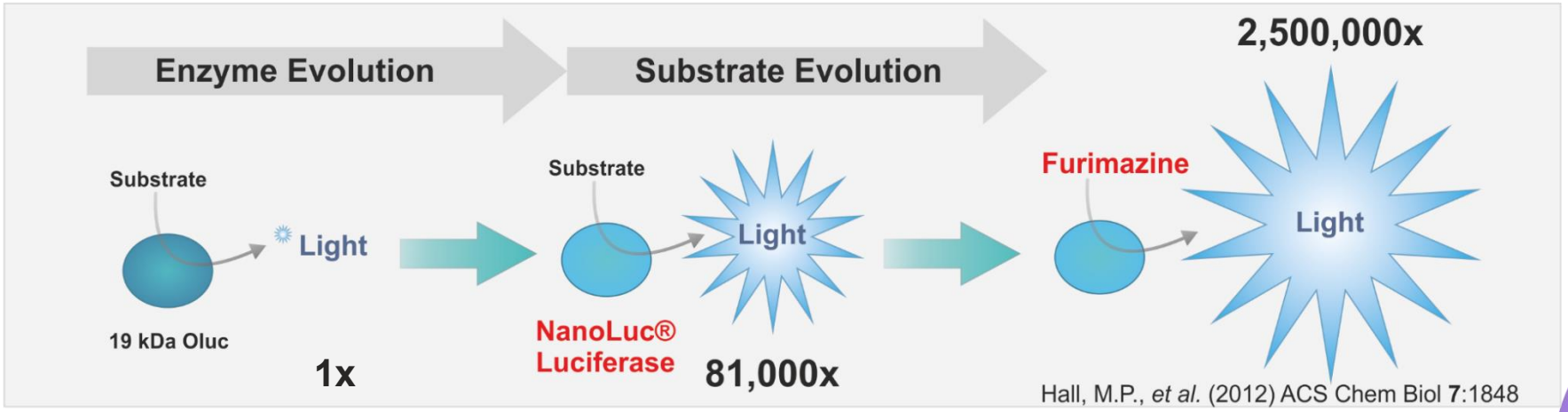
Oplophorus gracilirostris



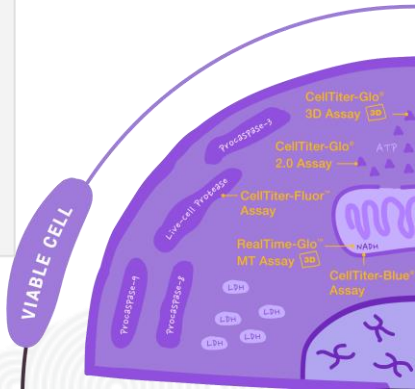
- *Oplophorus* luciferase 106 kDa
- 7x brighter than Rluc
- Glow luminescence
- Shimomura *et al.* 1978



- Catalytic subunit 19 kDa
- Light output & stability compromised
- Inouye *et al.* 2000 and 2007

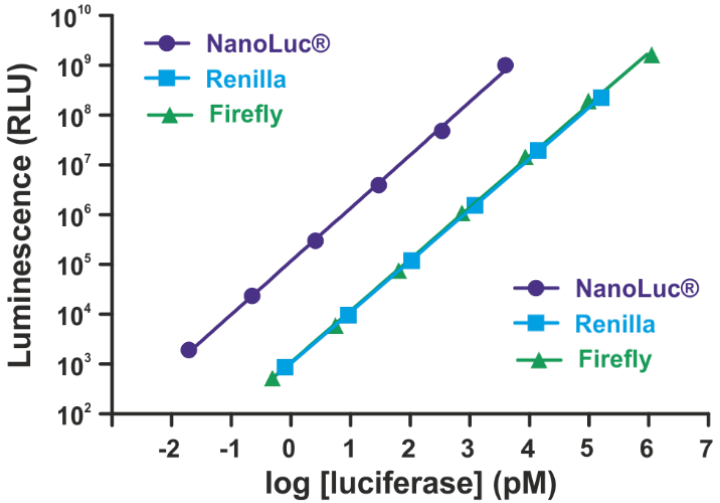


Hall, M.P., *et al.* (2012) ACS Chem Biol **7**:1848

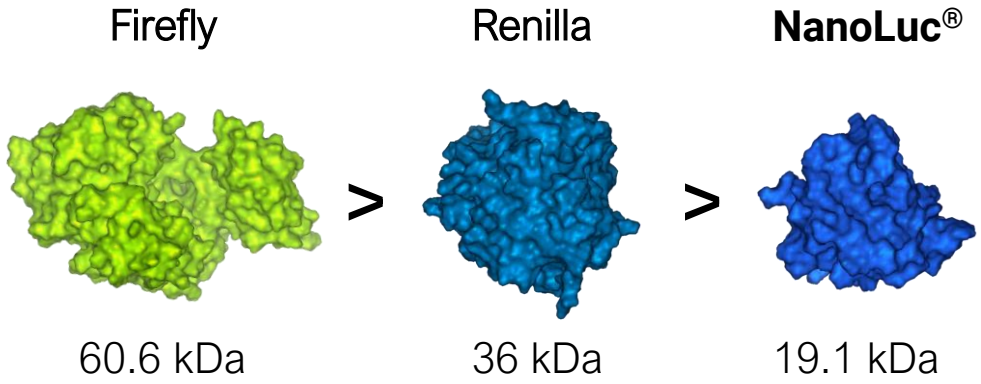


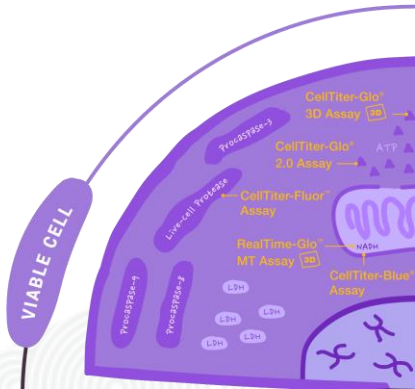
NanoLuc® Luciferase: A Bright and Small Reporter

Bright, Brighter, NanoLuc®

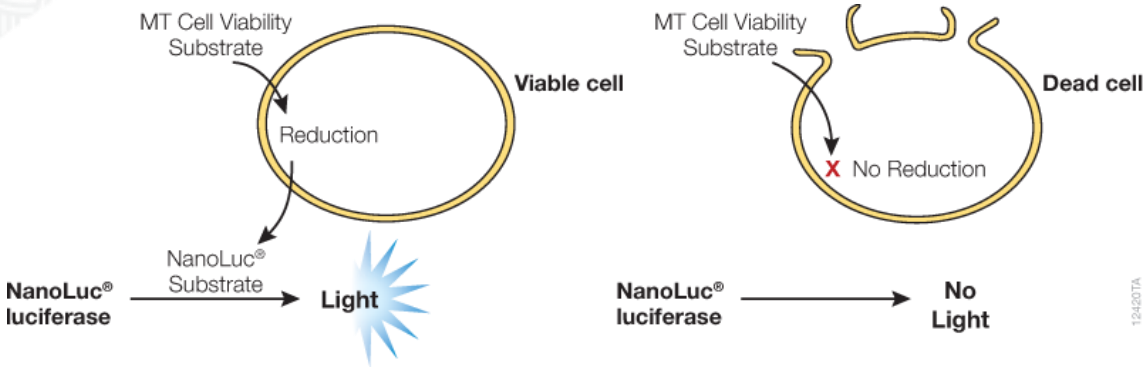


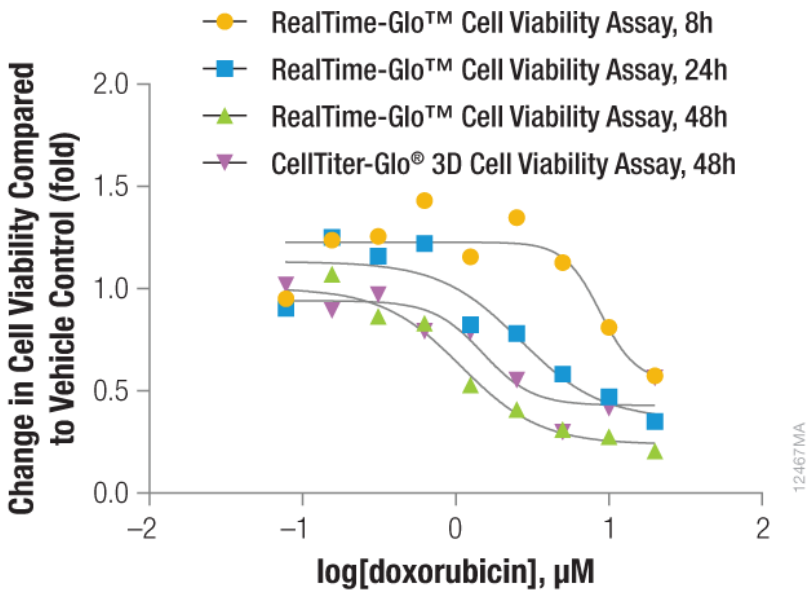
Small, Smaller, NanoLuc®

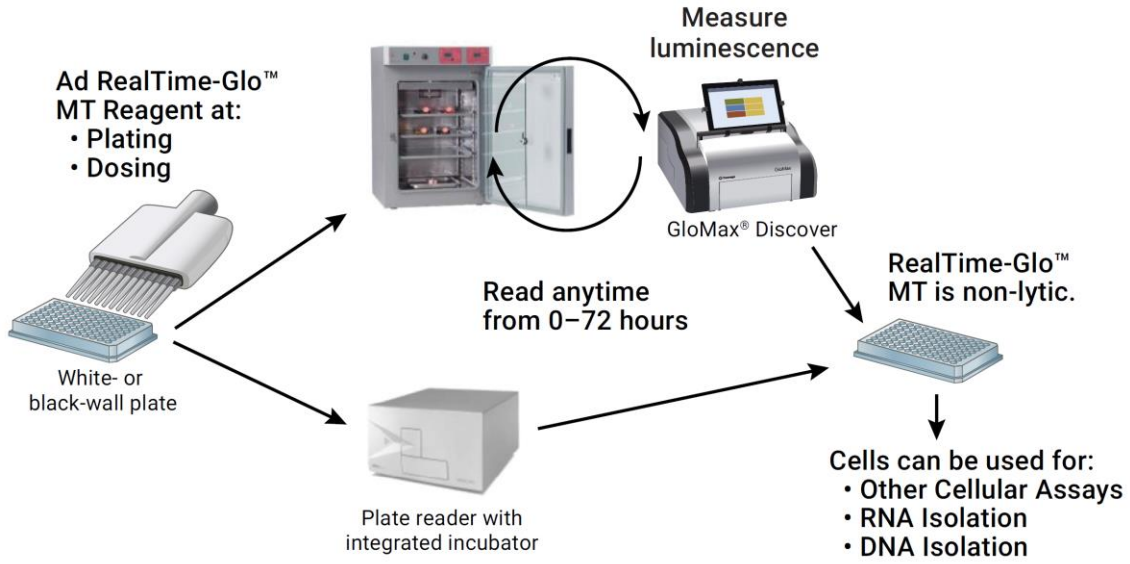




RealTime-Glo™ MT Cell Viability Assay



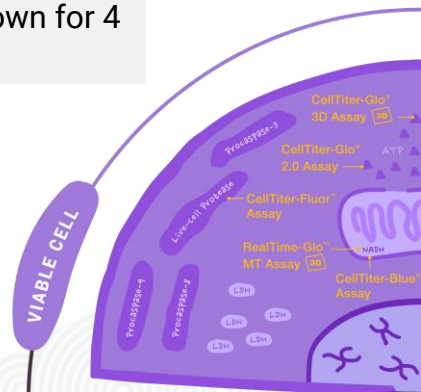




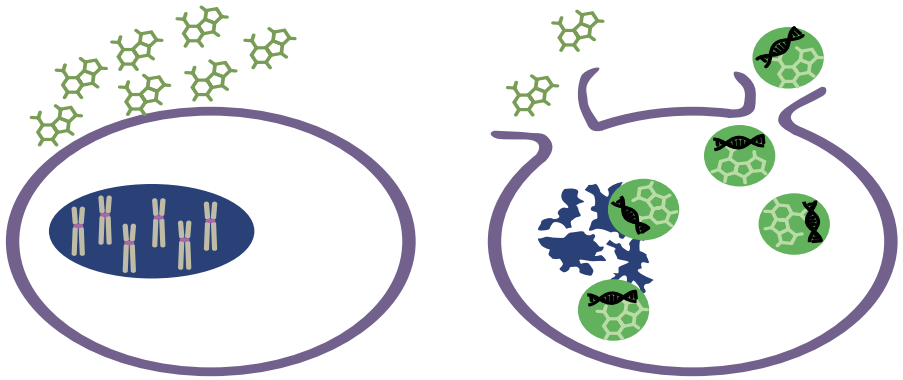
	RealTime-Glo™ Assay at 8 hours	RealTime-Glo™ Assay at 24 hours	RealTime-Glo™ Assay at 48 hours	CellTiter-Glo® Assay at 48 hours
EC_{50} Value (µM)	8.77	2.65	1.09	1.50

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

spheroid hydrogel organoid microfluidic

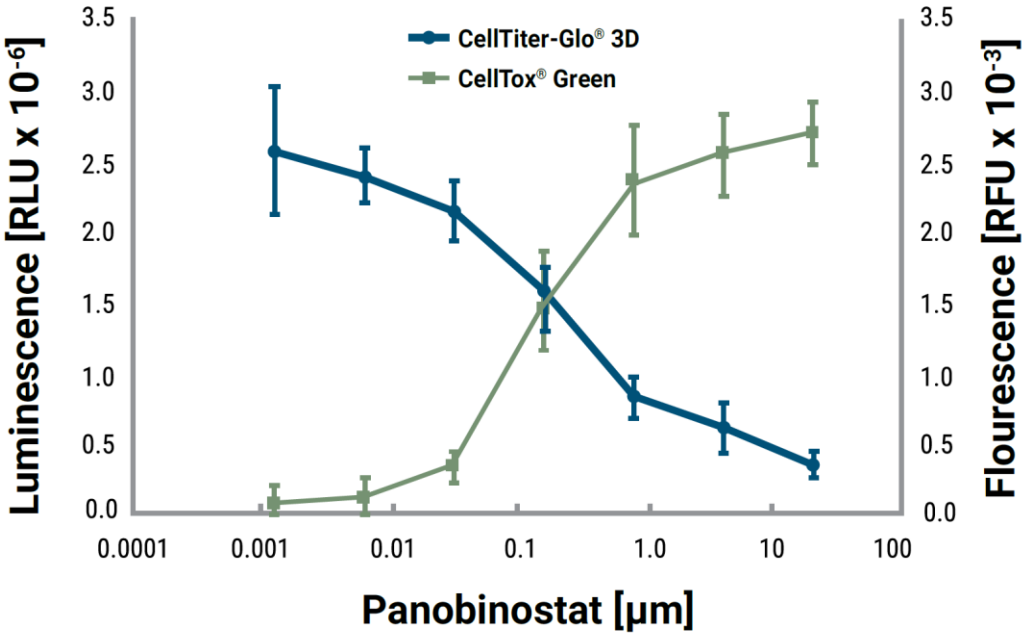


CellTox™ Green Cytotoxicity Assay

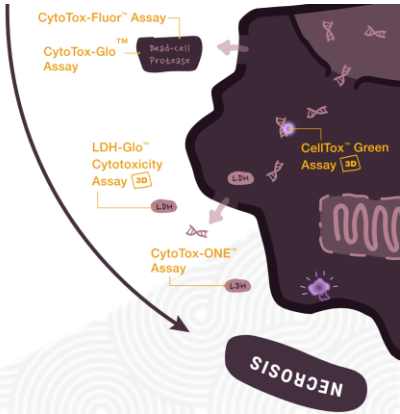


Low Fluorescence Viable Cells

High Fluorescence Nonviable Cells

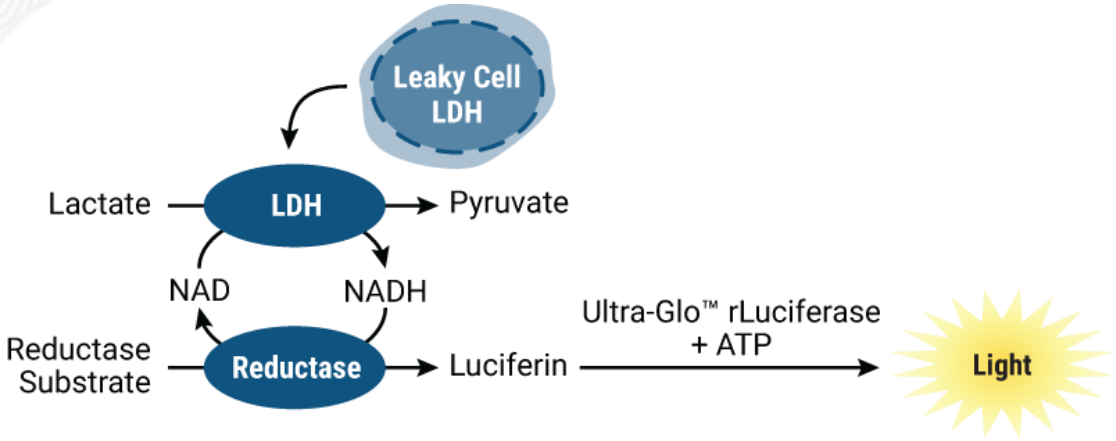


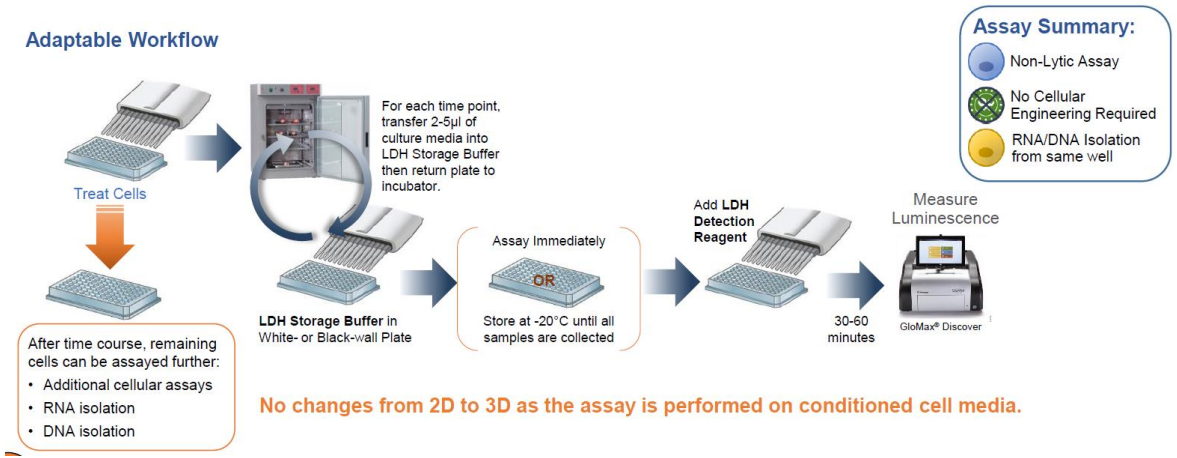
HCT116 colon cancer spheroids were grown for 4 days in a 96-well hanging drop plate



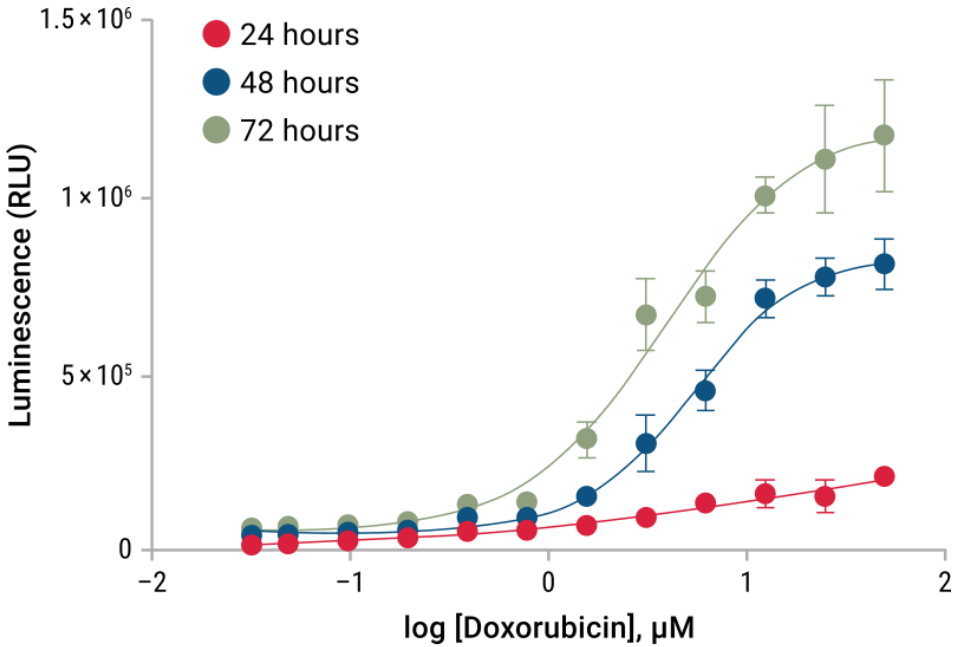
spheroid hydrogel organoid microfluidic

LDH-Glo™ Cytotoxicity Assay



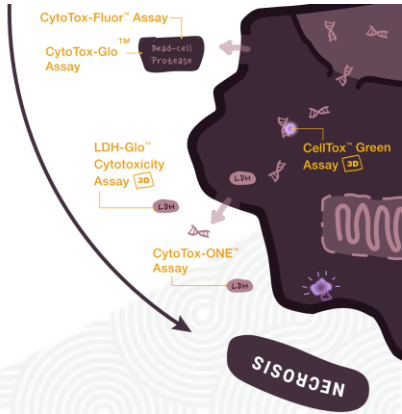


No changes from 2D to 3D as the assay is performed on conditioned cell media.

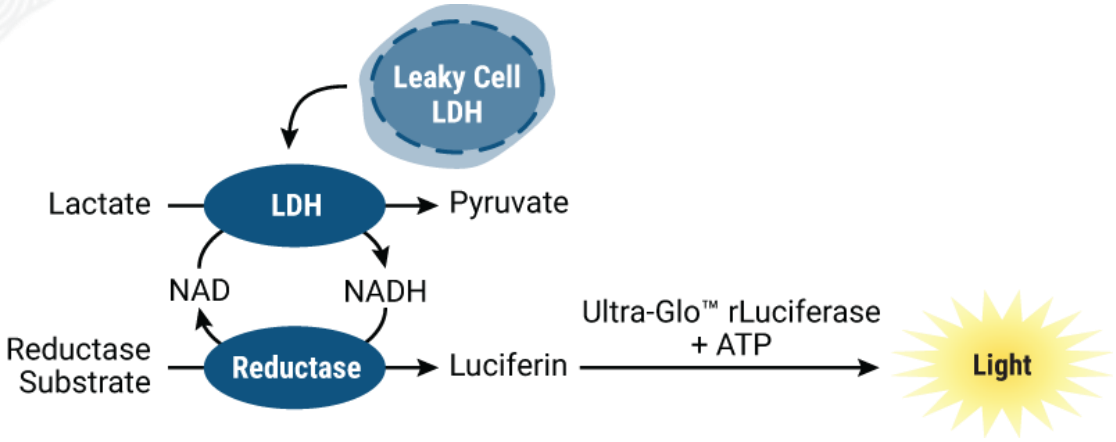


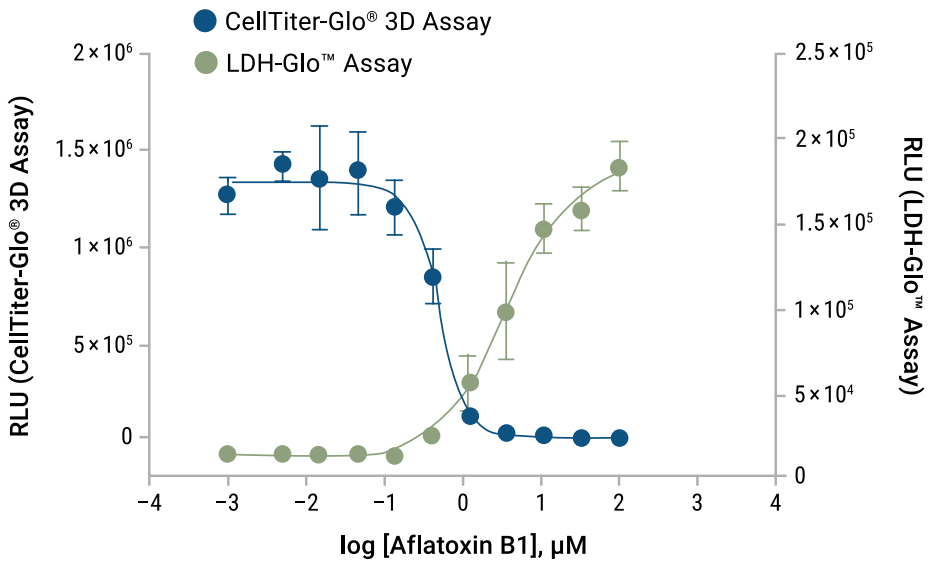
	24 hours	48 hours	72 hours
EC50	17.09	5.474	3.844

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



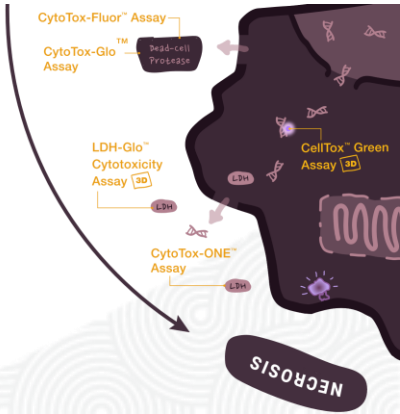
LDH-Glo™ Cytotoxicity Assay



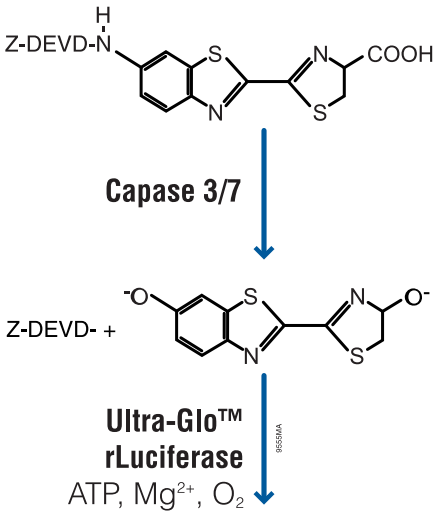


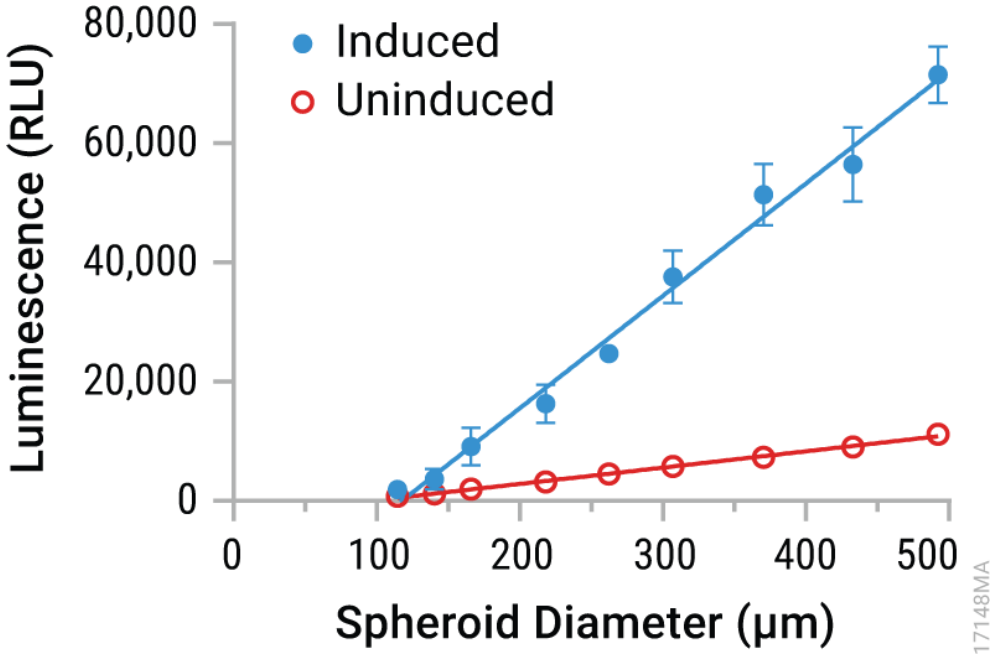
	LDH Assay	CTG 3D Assay
EC50	3.52	0.4999

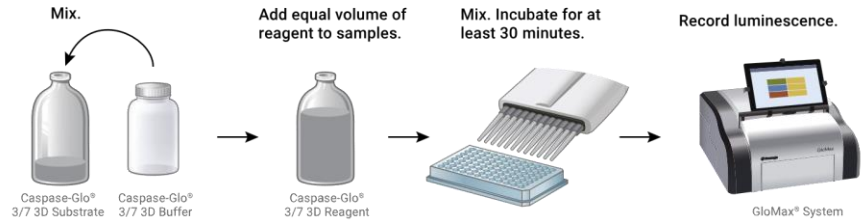
Human liver microtissue spheroids were treated with aflatoxin B1 for 48 hours.



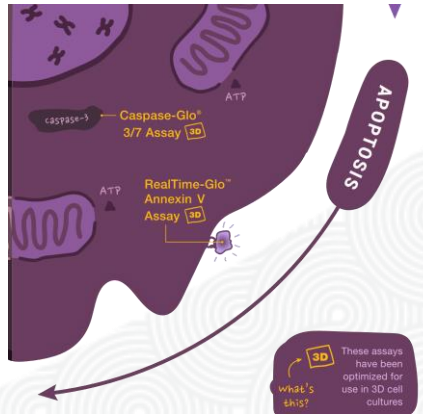
Caspase-Glo® 3/7 3D Assay



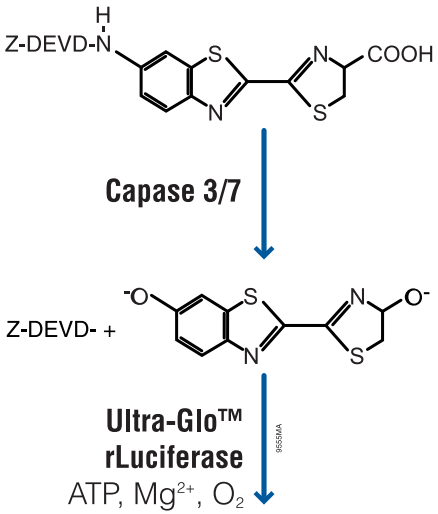


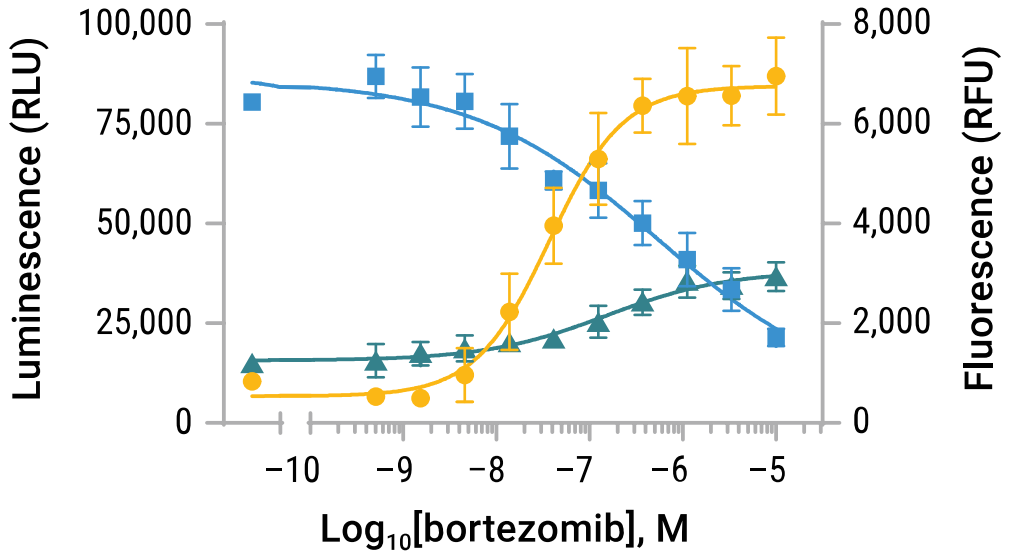


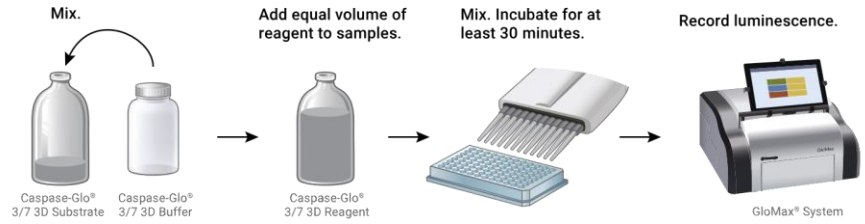
HCT116 cells were grown as spheroids in 96-well ULA plates (Corning)



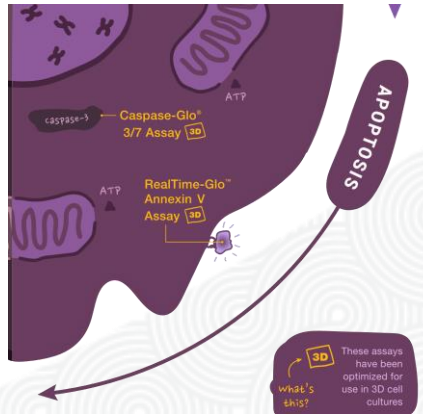
Caspase-Glo® 3/7 3D Assay



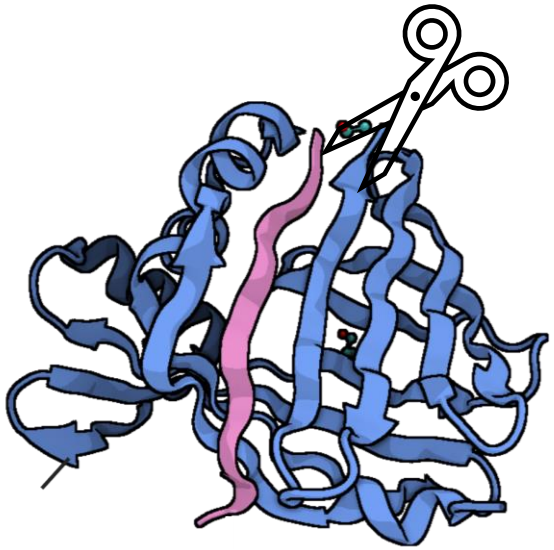




A549 spheroids (average diameter of 450µm)

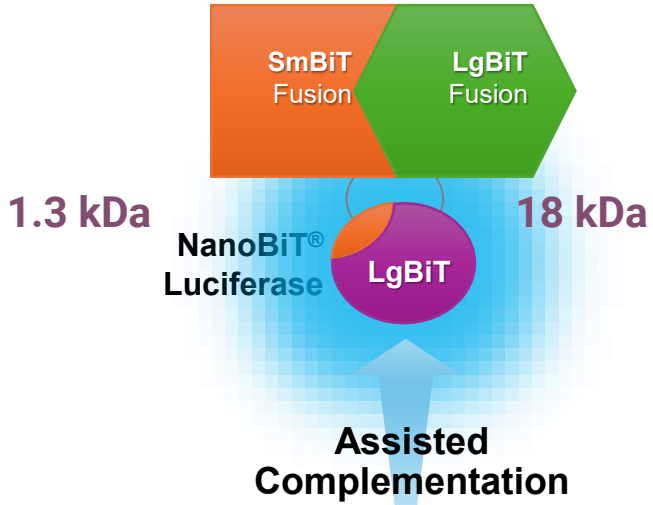


NanoLuc® Binary Technology (NanoBiT®)



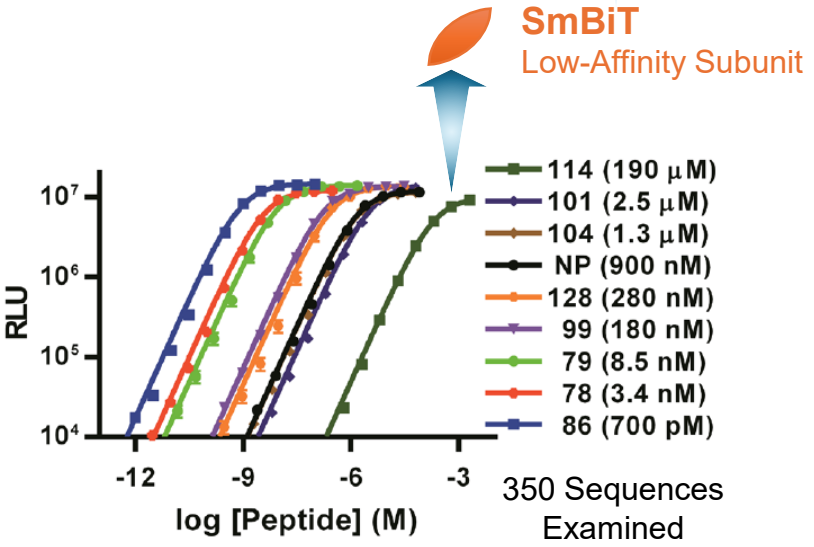
NanoLuc®

Protein:Protein Interactions



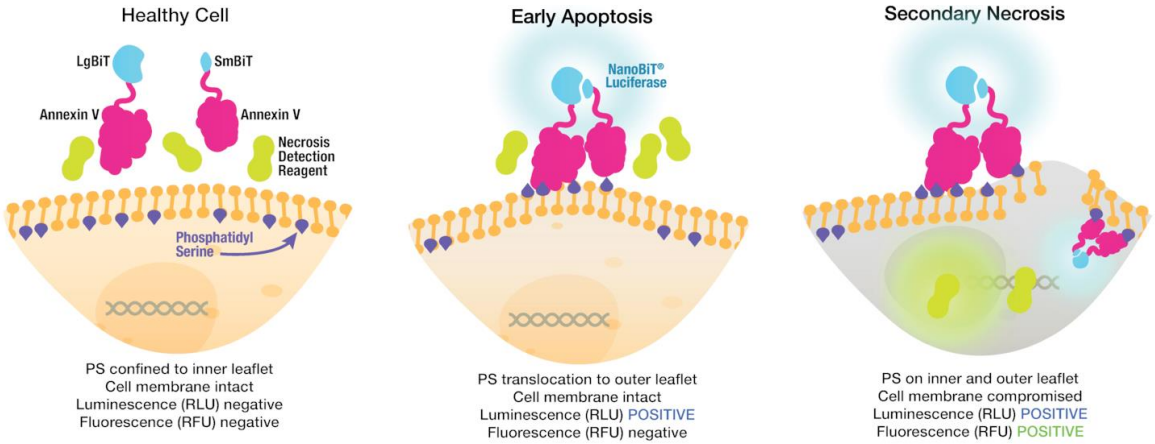
Small tag Size
minimal influence on fusion partner

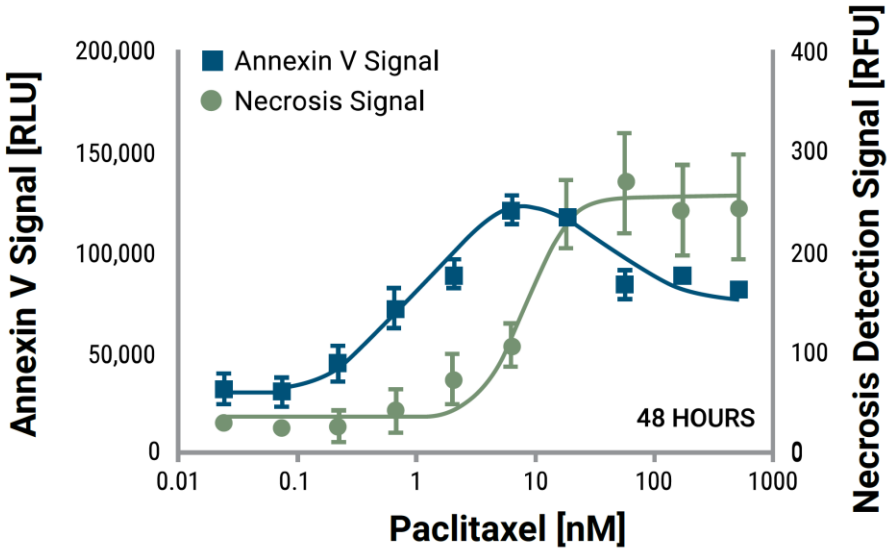
Bright signal upon complementation enables low expression levels



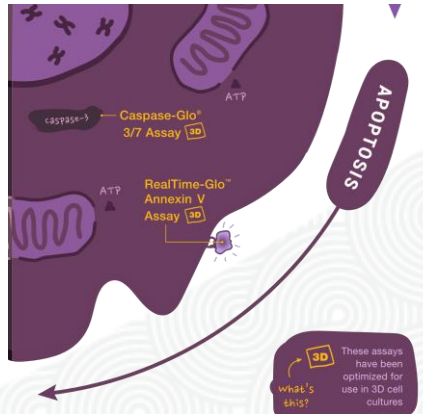
350 Sequences Examined

RealTime-Glo® Annexin V Apoptosis and Necrosis Assay

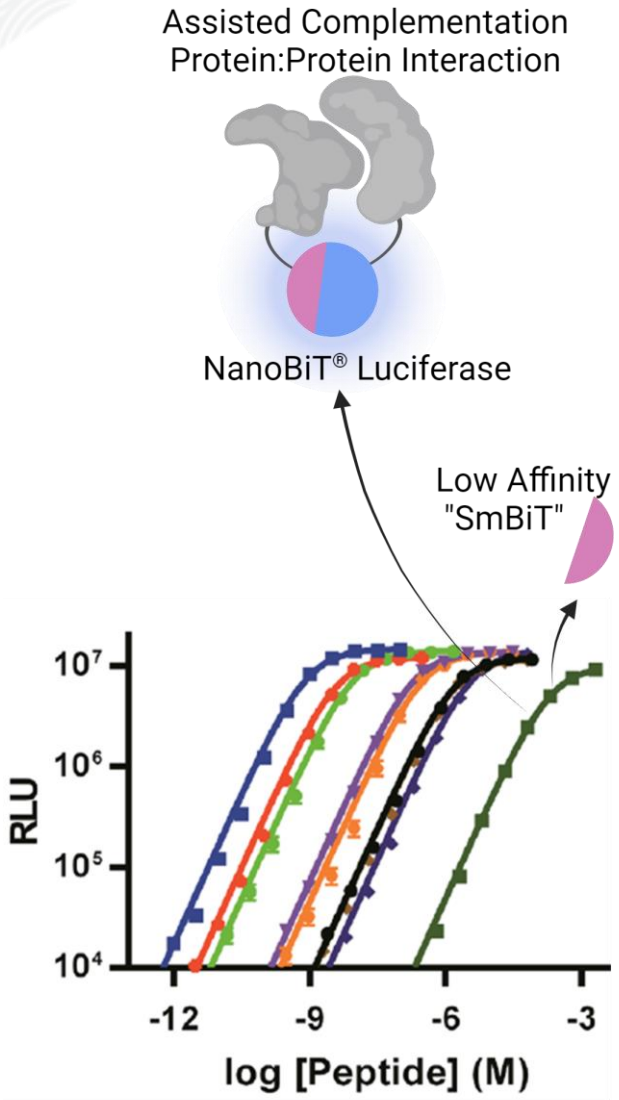


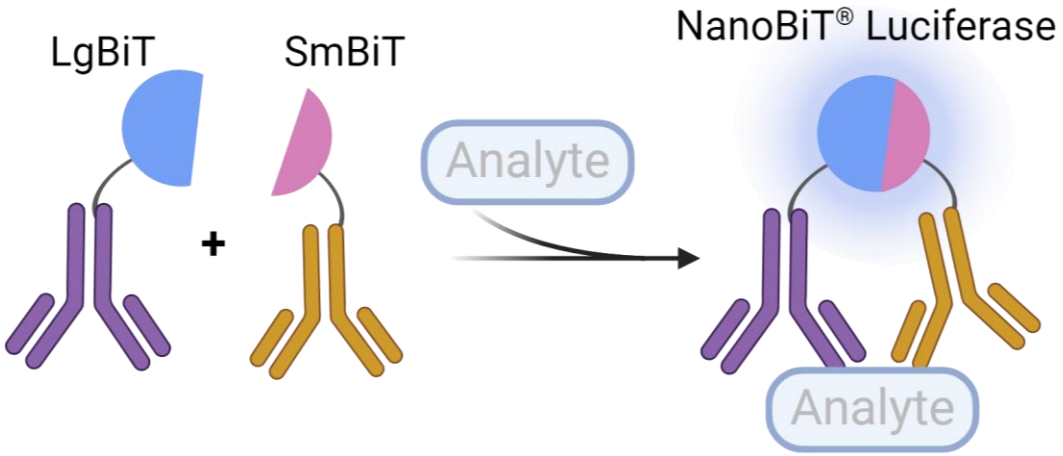


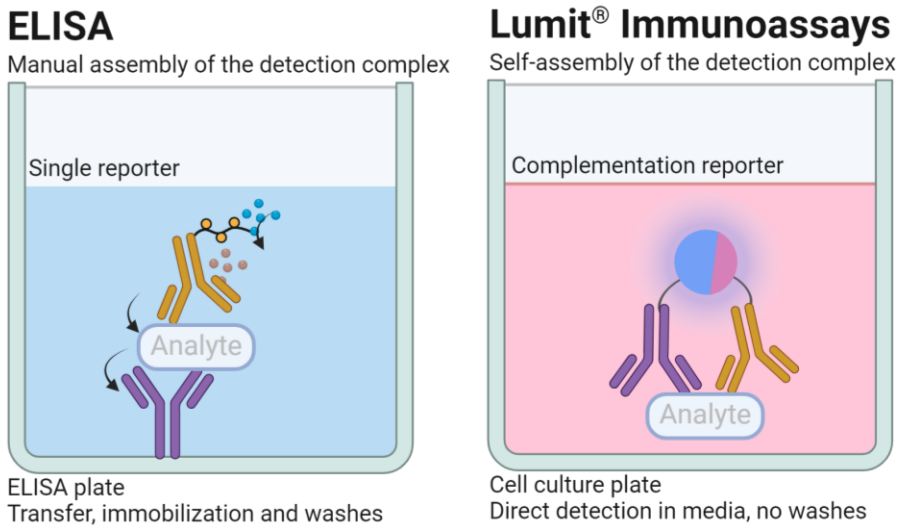
HepG2 spheroids were treated for 48 hours with different concentrations of paclitaxel



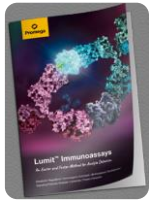
Lumit® Immunoassays: Detect Analytes and Molecular Interactions



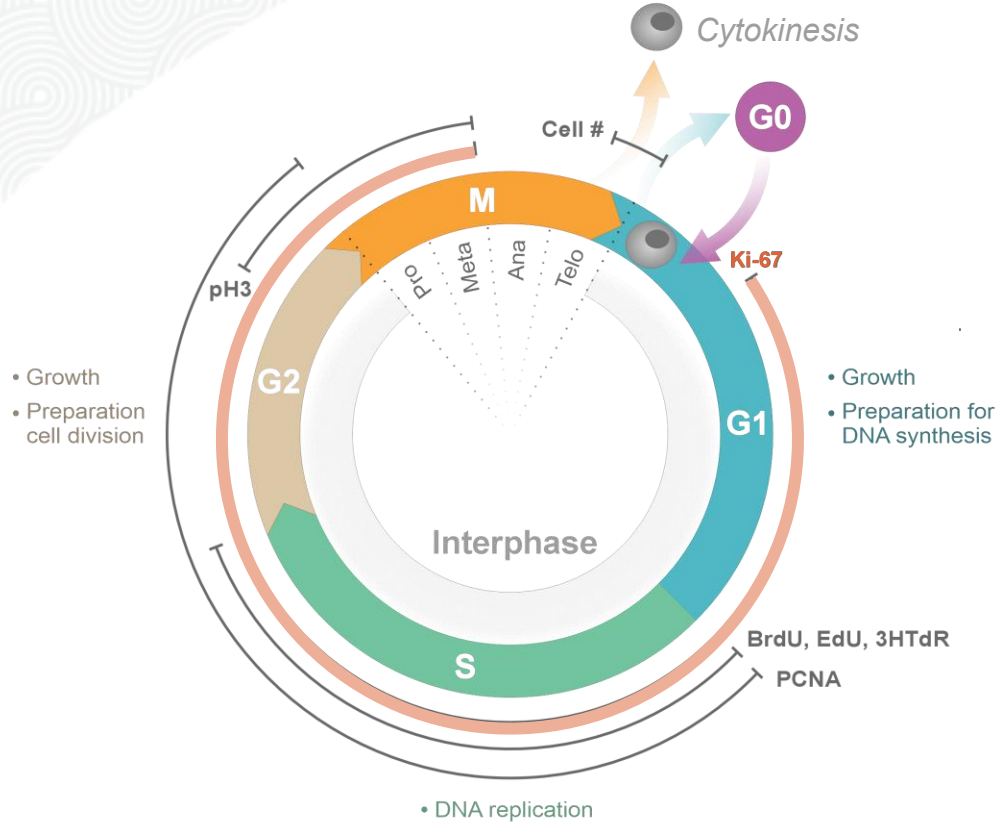


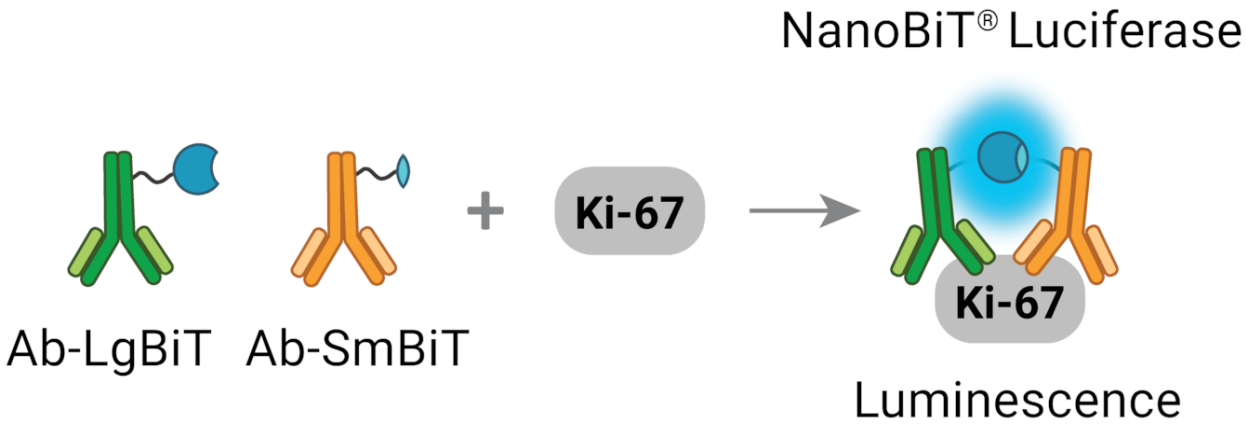


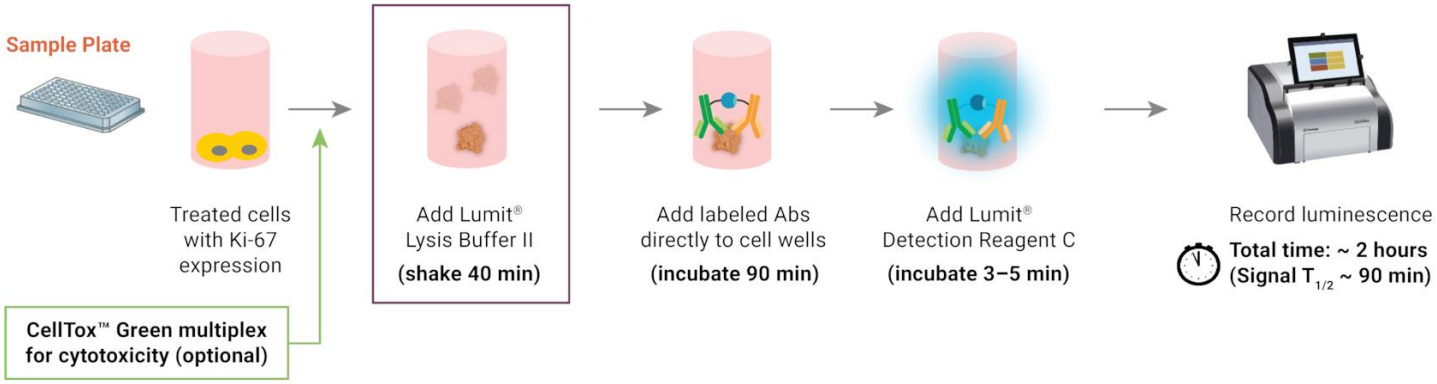
Lumit™ is a platform technology.
Learn more in this eBook:



Lumit® hKi-67 Immunoassay for Cell Proliferation



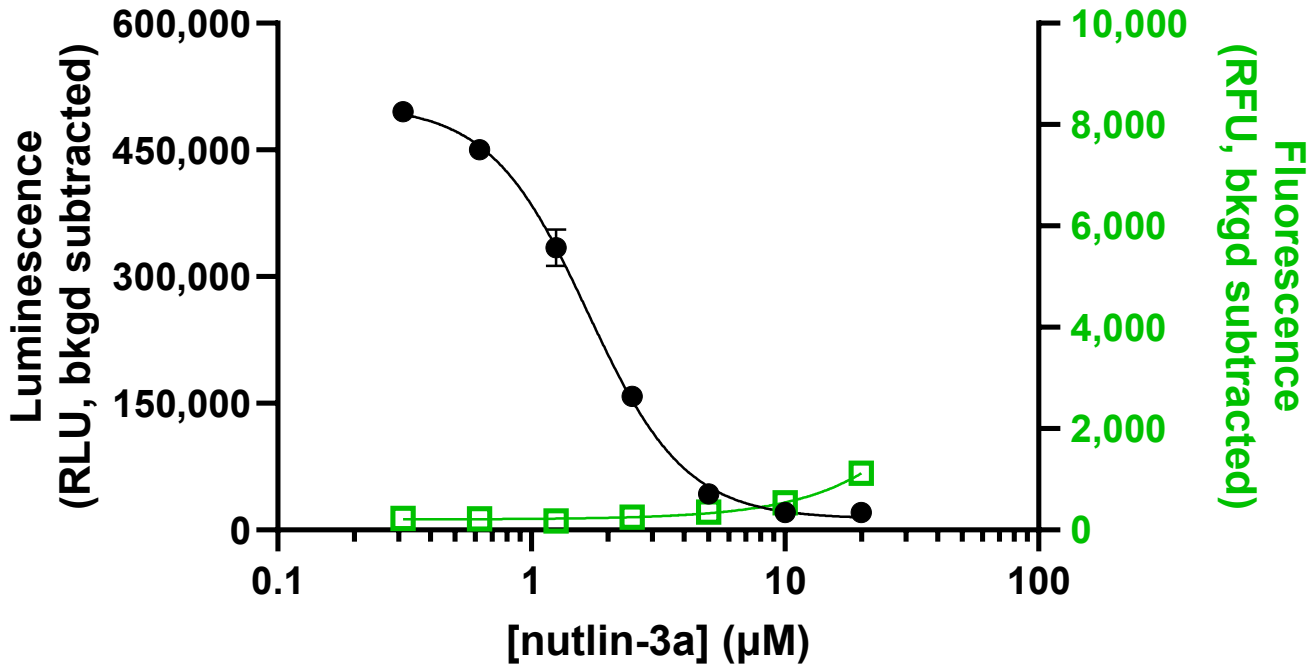




- Expressed in proliferating cells
  - Expressed in G1, S, G2 and M cell cycle phases
  - Ramps up from G1 until peaks early in M phase
- Absent in resting, non-dividing cells (G0) (quiescent, senescent, or terminally differentiated)

Lumit® hKi-67 Immunoassay for Cell Proliferation

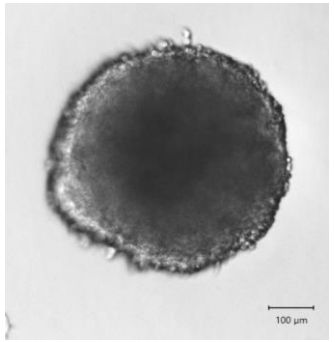
hKi-67 expression



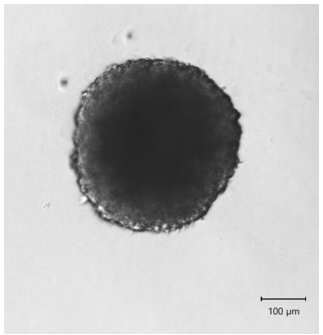
HCT116 cells (1000/well) were plated cultured 72h to form **spheroids** (**~400µm**), then treated 48h with increasing concentrations of **nutlin-3a** before assay.

CellTox Green followed by **Lumit hKi-67**

CellTox Green Cytotoxicity

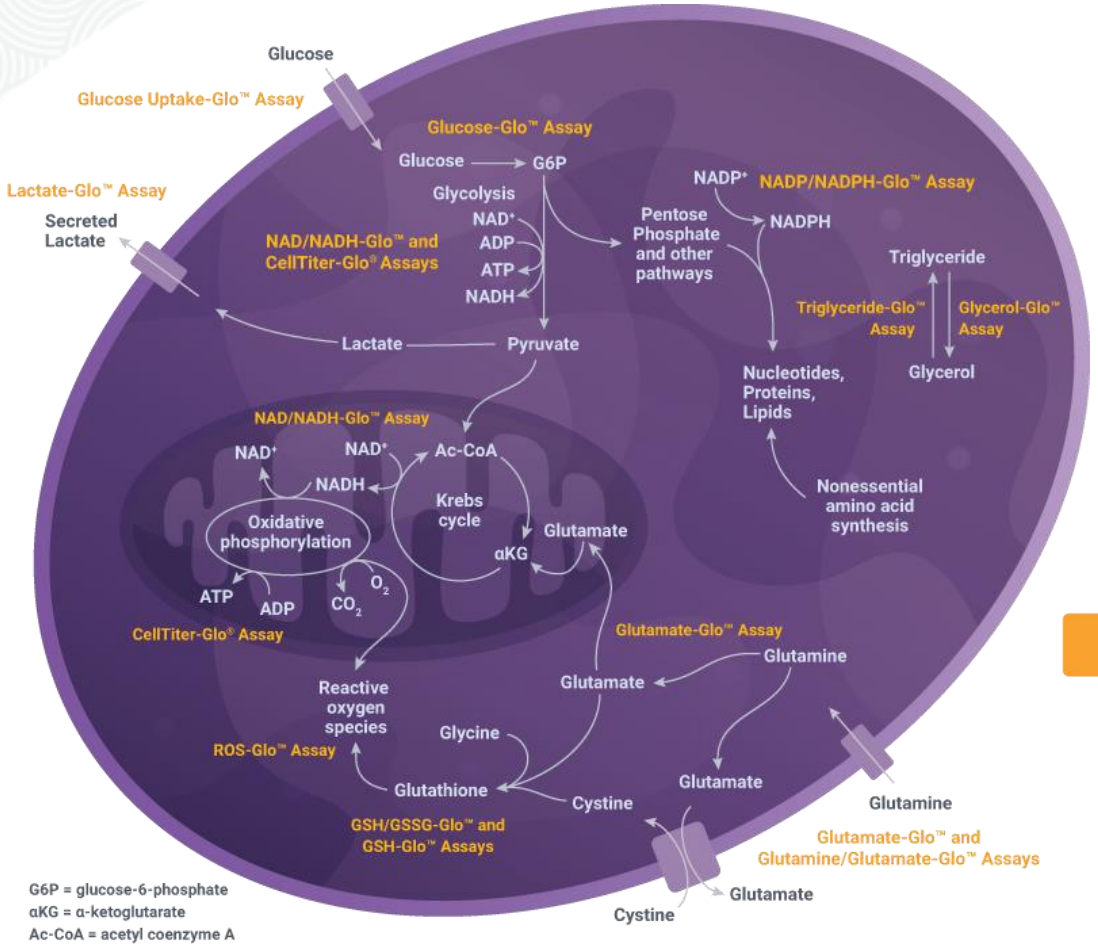


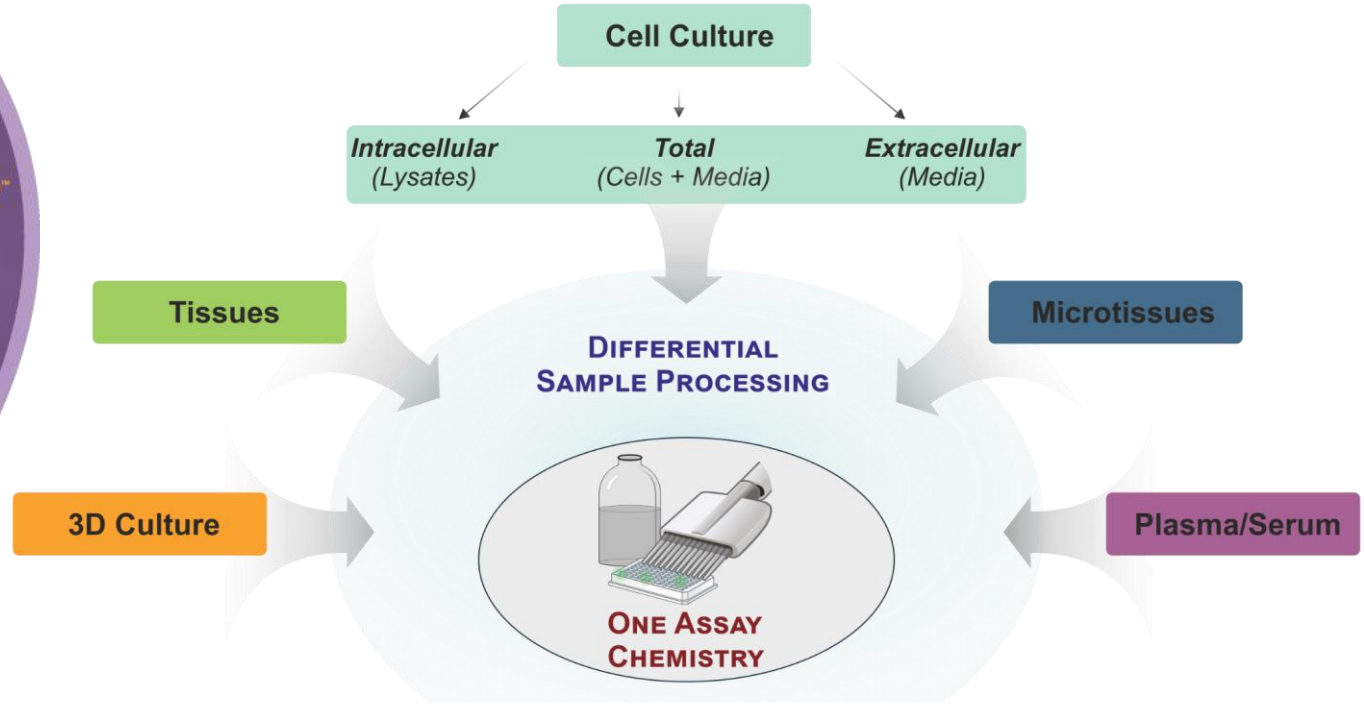
Untreated



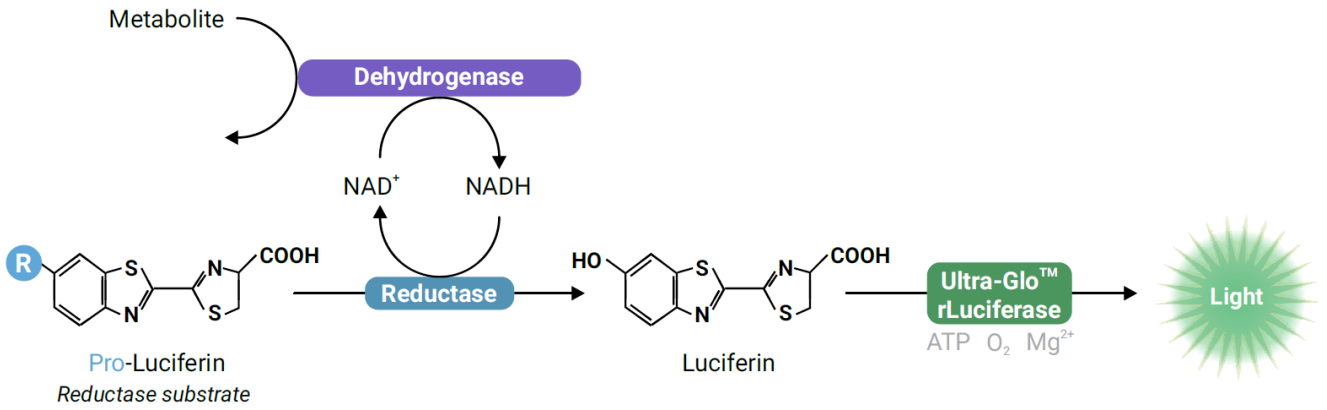
Nutlin-3a (20µM, 48h)

Cell Energy Metabolism



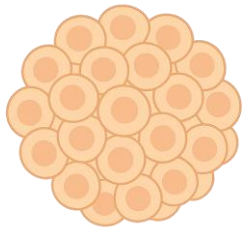


Metabolite Assays – One Reaction to Rule Them All



Glucose Metabolism	Amino Acid Metabolism	Lipid Metabolism	Mitochondrial Function	Cofactors	Oxidative Stress
Glucose-Glo™ S	Glutamate-Glo™ S	Triglyceride-Glo™ S	ATP	NAD/NADH-Glo™	ROS-Glo™
Lactate-Glo™ S	Glutamine/Glutamate-Glo™ S	Glycerol-Glo™ S	Pyruvate-Glo™ S	NADP/NADPH-Glo™	GSH-Glo™
Glucose Uptake-Glo™ (2DG6P)	BCAA-Glo™ S	Cholesterol/Cholesterol Ester-Glo™ S	Malate-Glo™ S	NAD(P)H-Glo™	GSH/GSSG-Glo™
Glycogen-Glo™		BHB-Glo™ S			

Downstream Applications

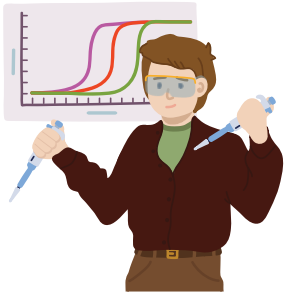


Spheroid

Microplate readers or Bioluminescent Imager

Manual or automatic nucleic acid isolation

qPCR or qRT-PCR



Capillary Electrophoresis

Promega Bioluminescence Instrument Portfolio

MyGlo™ Reagent Reader

- ✓ Luminescence with limited assays

GloMax® Navigator

- ✓ Luminescence
- ✓ +Injectors

GloMax® Explorer

- ✓ Luminescence
- ✓ Fluorescence
- ✓ Vis Absorbance
- ✓ Heating
- ✓ Shaking

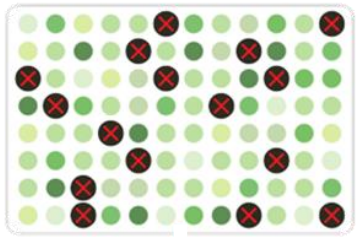
GloMax® Discover

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

GloMax® Galaxy

- ✓ NanoLuc Technology based Bioluminescence Imager

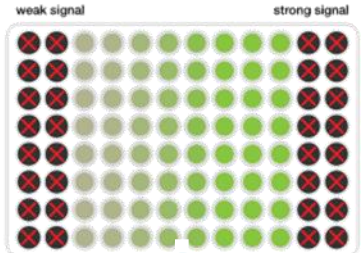
Common Problems that Users Encounter



"I can't detect samples below X # of cells"

Poor sensitivity leads to assay limitations; false negatives

PubHub article



"The signals from my cells are too close to background"

Narrow detection range limits usable range of your assay

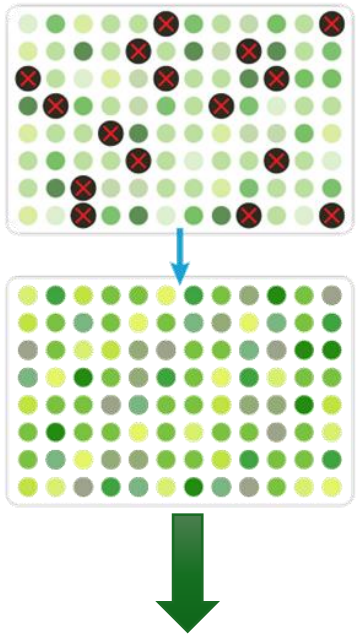
PubHub article

"I get higher signals that I expect from certain wells. Are these real?"

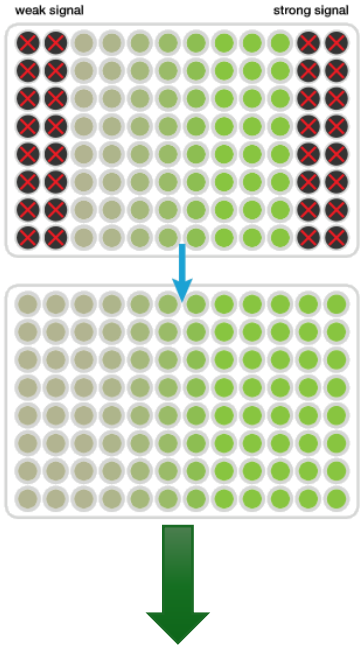
High cross-talk create misleading results; careful plate layout

PubHub article

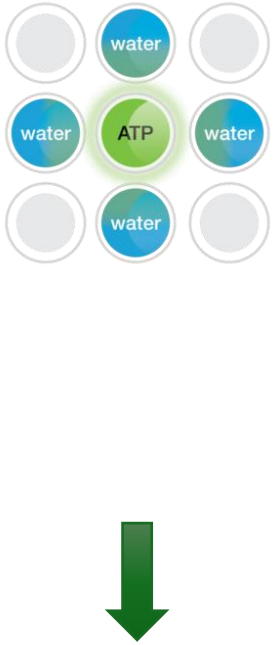
Luminometer Choice Does Make a Difference!



Superior sensitivity so you don't miss hits



Broad detection range so you don't limit your experiments



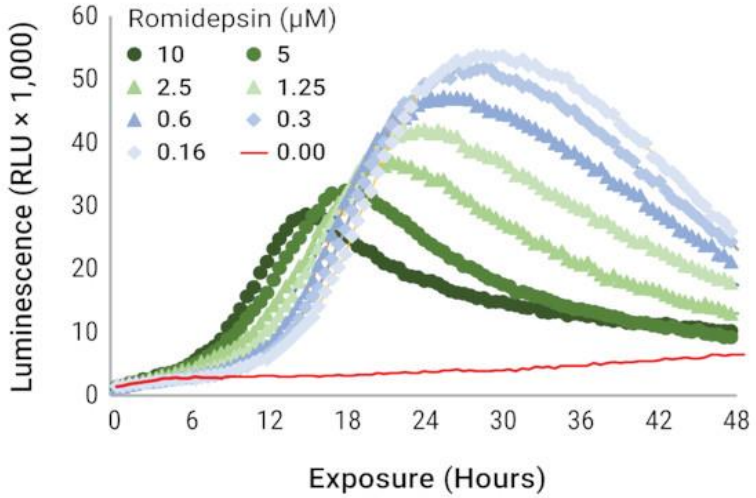
Masking so crosstalk doesn't mislead your results

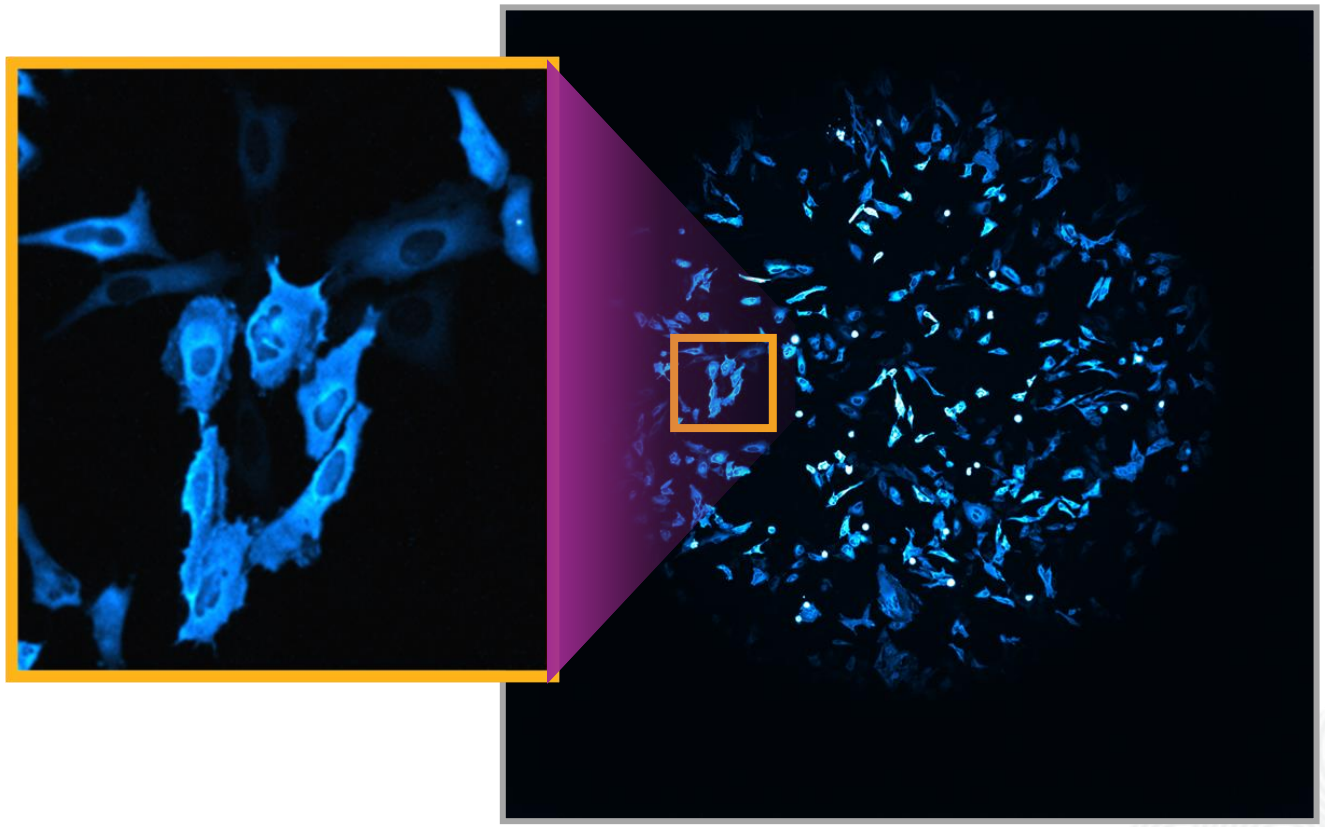
GloMax® Systems

Live Cell Microscopy

Plate Reader

Live Cell Microscopy





"Is the response occurring in all cells?"

"What is the subcellular origin?"

"Are there responders and non-responders?"

"Is there an impact on cell morphology?"

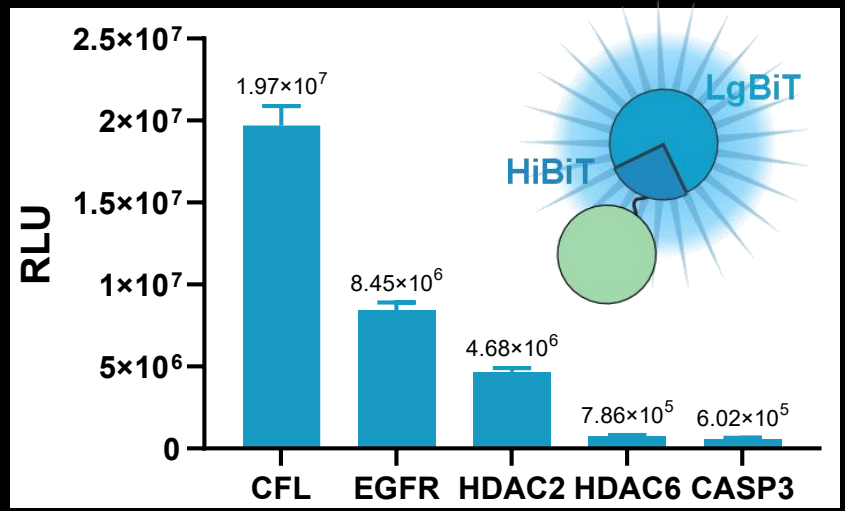
GloMax® Galaxy Bioluminescent Imager

Dimensions (W × H × D)	14.8 inches × 18.8 inches × 21.0 inches (37.3cm × 47.7cm × 53.3cm)
Weight	Approximately 62lb (25kg)
Power Requirements	100–240V AC, 50/60Hz
Camera	Retiga E7 (CMOS), 4.5 × 4.5µm pixel size, 12-bit (0–4,095 gray scale)
Wavelength Range	400–750nm
Optics	Nikon CFI Plan Apochromat Lambda D 20X/0.75 NA Custom 100mm focal length Tube Lens (~10.35X System Magnification)
Image pixel size	1 pixel = 0.435µm

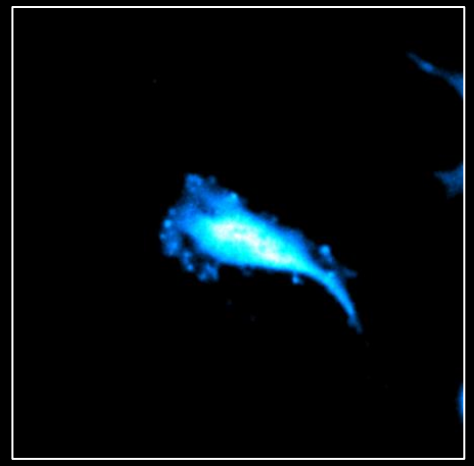
LUMINESCENCE	FLUORESCENCE	BRIGHTFIELD
Protein dynamics & localization	Cellular reference markers	Cell Morphology

Imaging Low Abundance Endogenous Proteins

- HiBiT-encoding sequence was inserted to genomic locus via CRISPR/Cas9 in HeLa cells
- Tagged proteins are expressed under control of native promoter
- NanoBiT® luciferase is formed by ectopic LgBiT expression
- Detection with the Nano-Glo® Live Cell Assay System

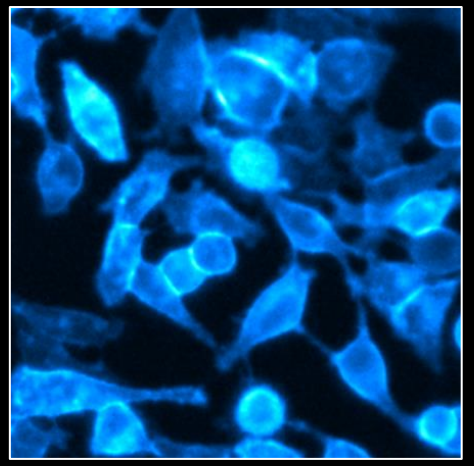


Cofilin



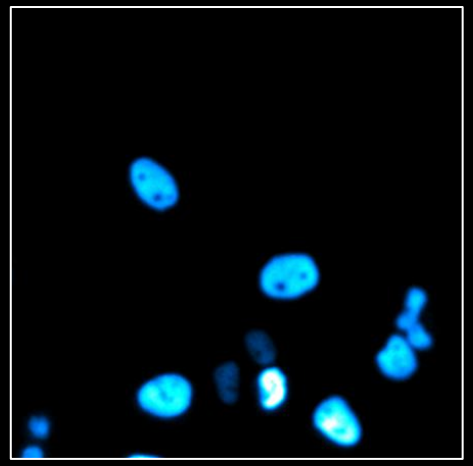
1-minute exposure

EGFR



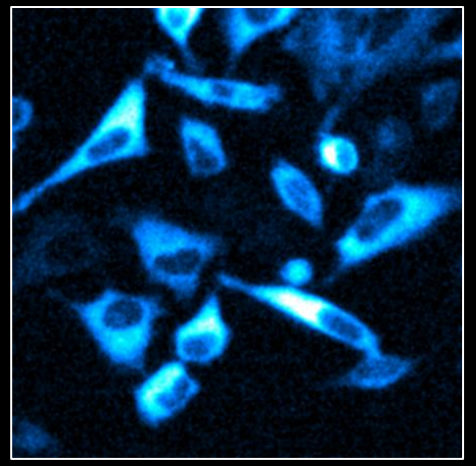
1-minute exposure

HDAC2



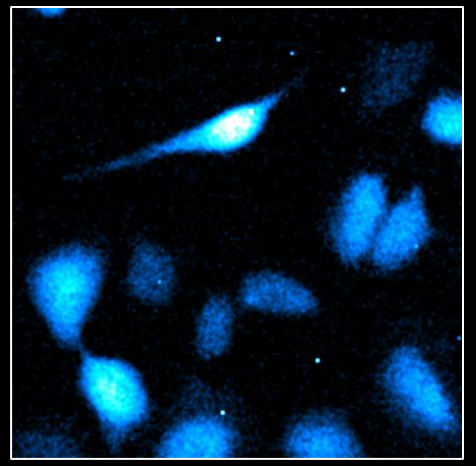
1-minute exposure

HDAC6



Low Expression
~3-minute exposure

CASP3

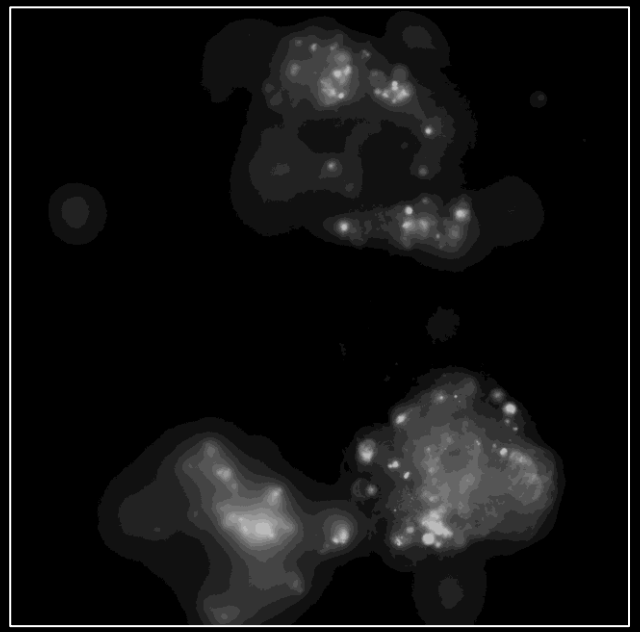


Very Low expression
~5-minute exposure

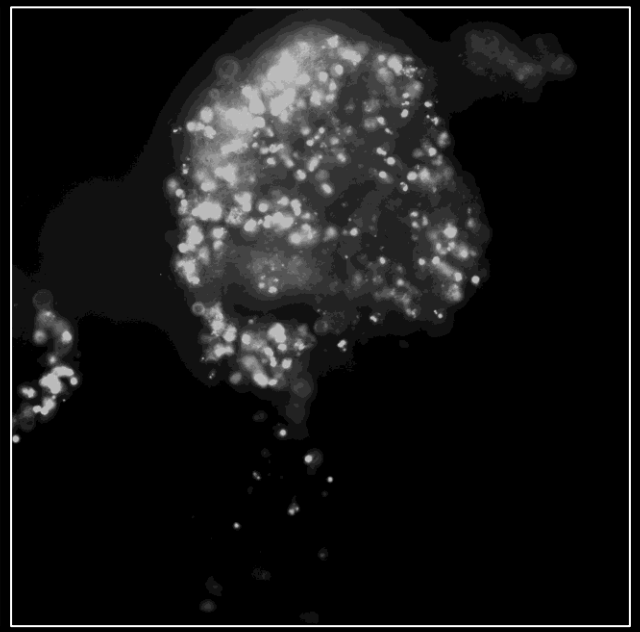
Chemotherapy treatement of patient-derived CRC organoids

Imaging of CellTox-Green (Fluorescence) after 3 days of treatment (DNA dye labels necrotic cells)

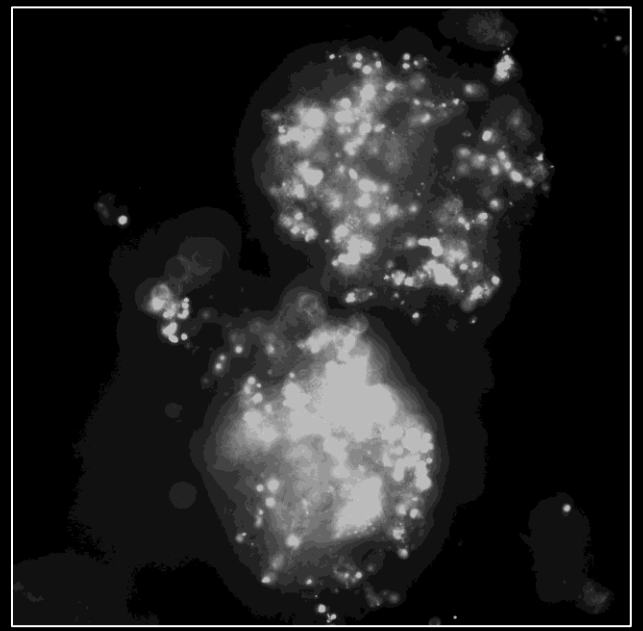
Control



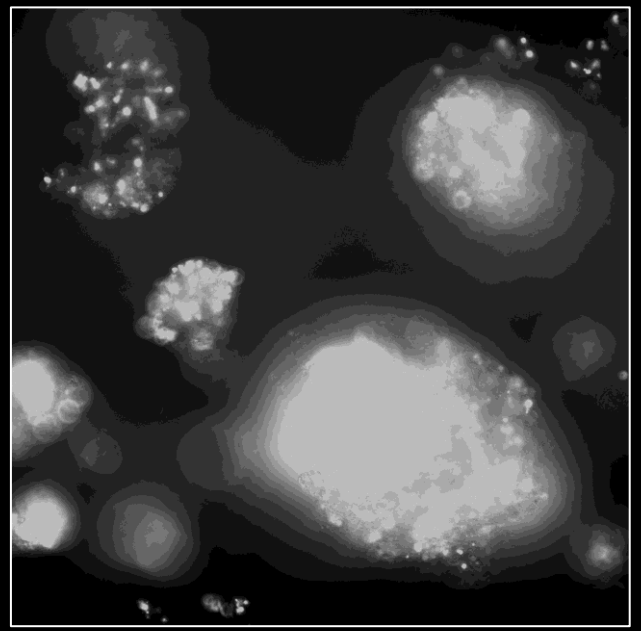
10nM



25nM

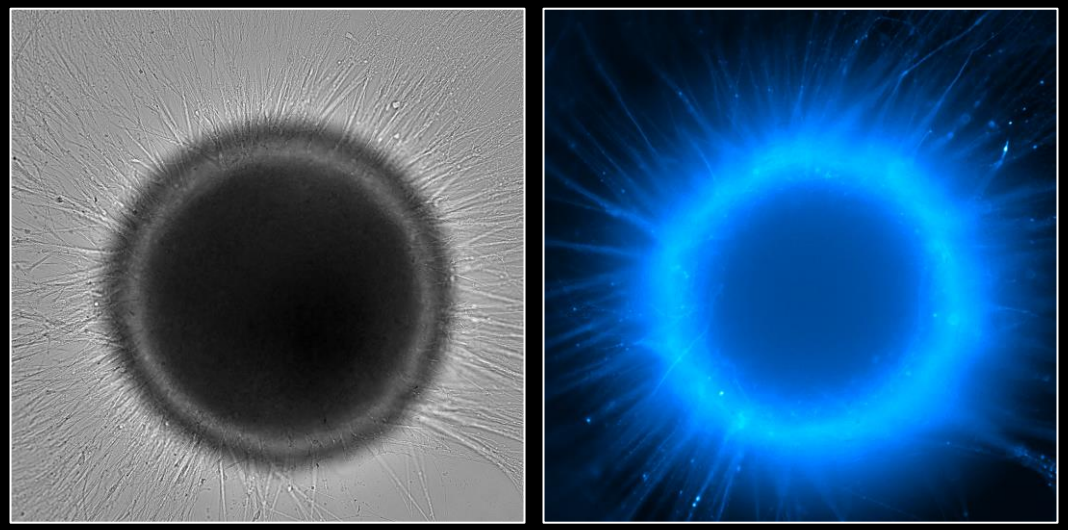


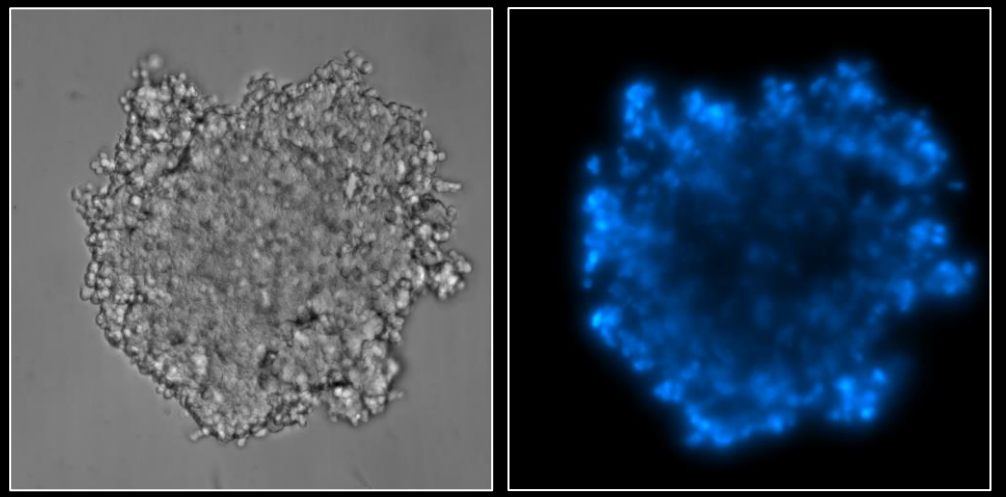
50nM



Imaging 3D models on the GloMax® Galaxy

- Neuropile visible extending from neurosphere, imaged on day 13 after culturing
- NanoGlo® Live Cell Substrate
- 5 second exposure

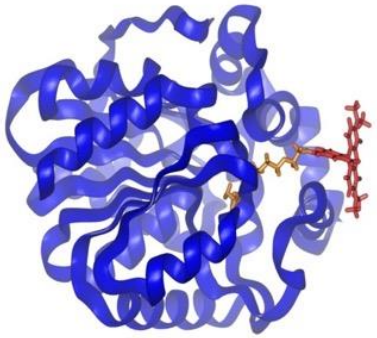




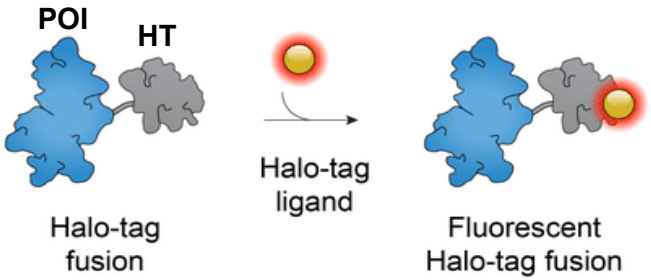
- 36-hour old HTC-116 spheroid stably expressing NLuc generated via Lentiviral transduction
- 10% of cells expressing NLuc provides clean images with distinguishable individual cells
- Spheroids incubated with 20 µM furimazine for 15-20 min prior to imaging
- 30 second exposure

Non-Destructive Methods for Monitoring 3D Cultures in Real-Time HaloTag

HaloTag- Fluorescent

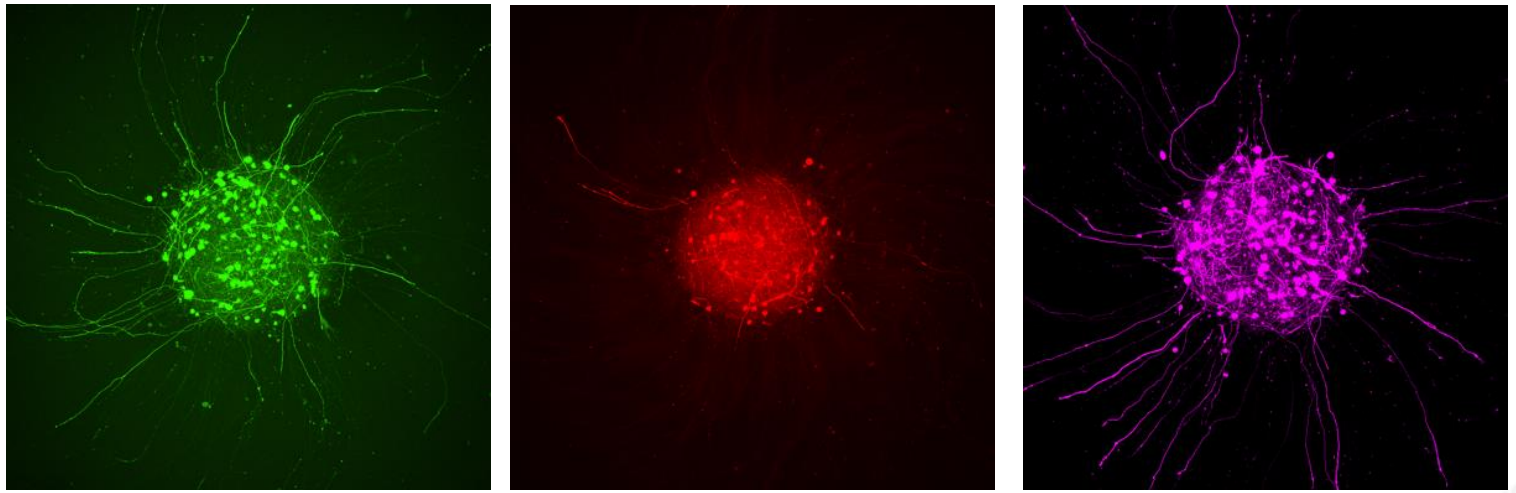


33KDa

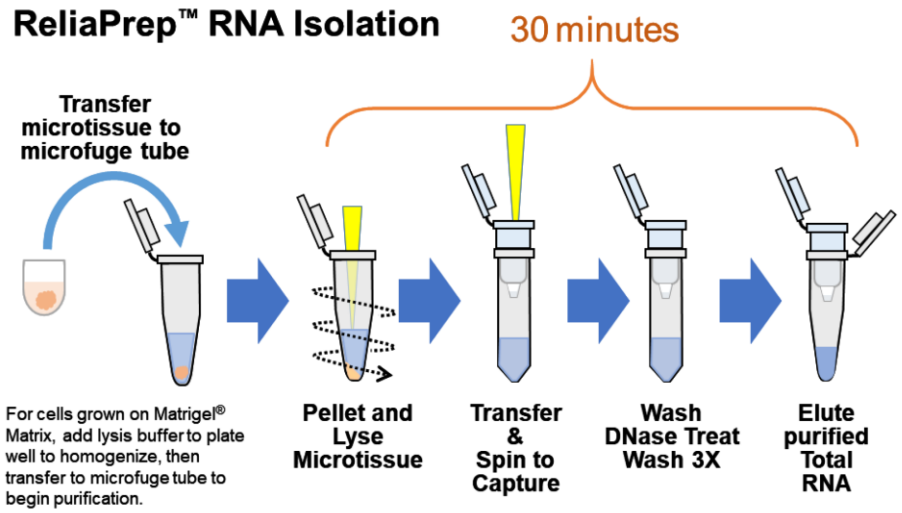


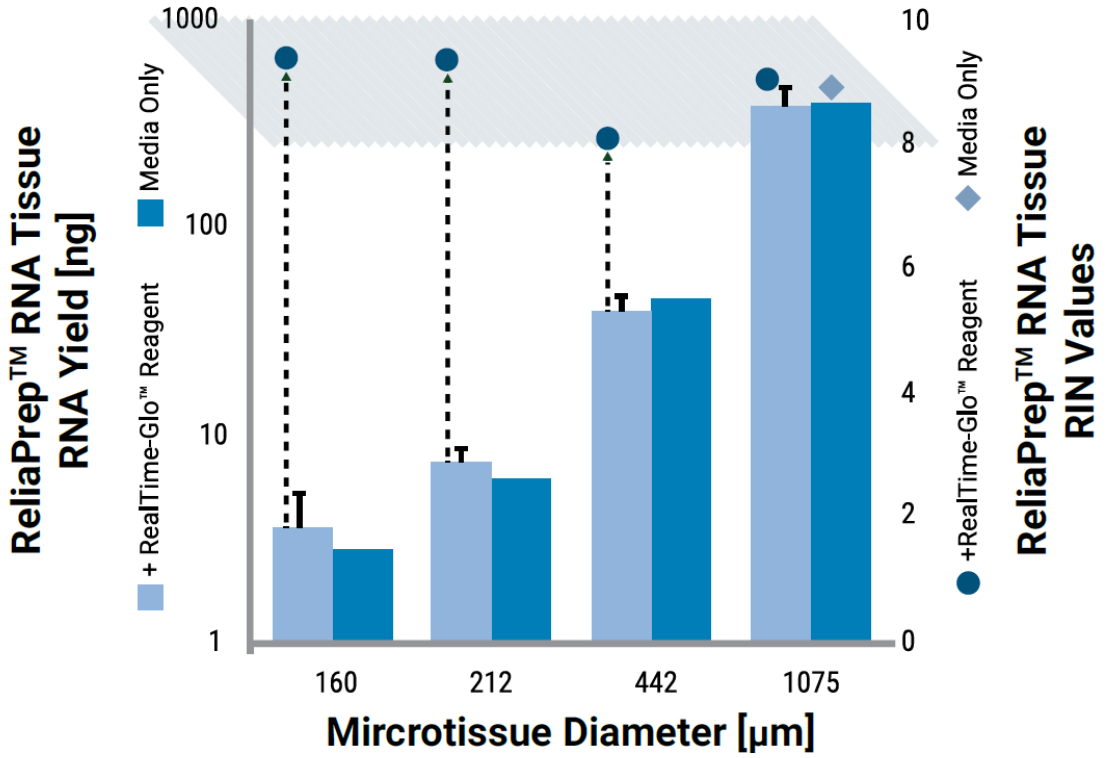
- Monomeric protein, 33KDa
- ***HaloTag ligands***: available in different colours on the spectrum with increase photostability, brightness and flurogenicity
- Live cell imaging, protein purification, PPI, TPD

JF503 **JF549** **JF646**



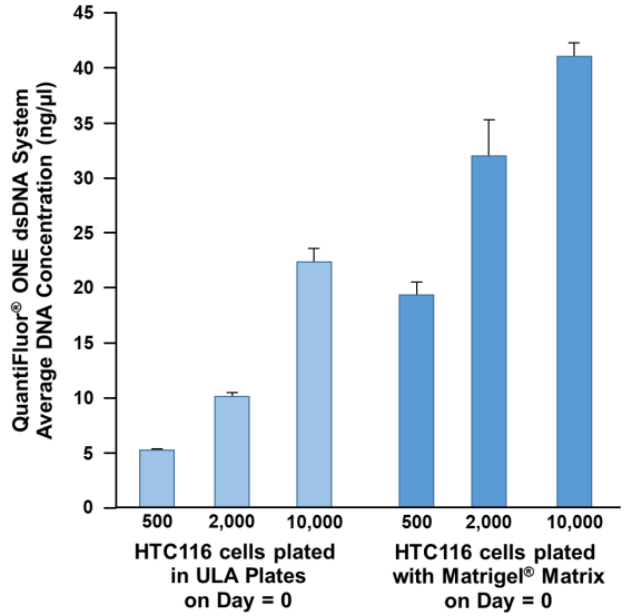
Manual RNA Isolation from 3D Cultured Cells





Maxwell® RSC Systems – Automate Your Workflow

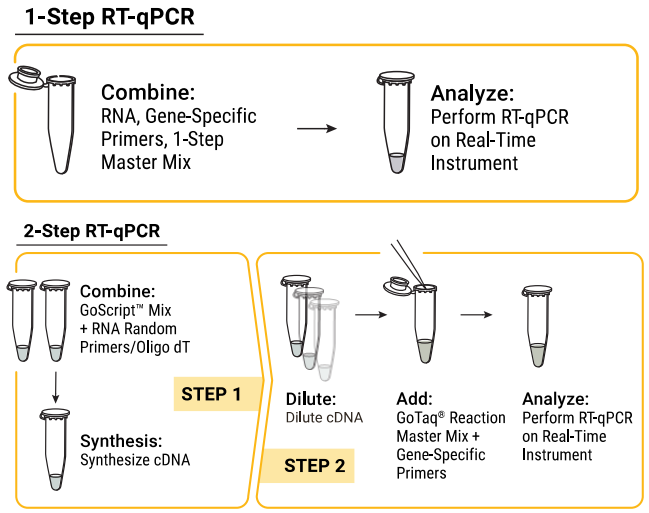
- Suitable for various downstream applications
- Purification from multiple sample types
- Prefilled cartridges and preinstalled methods
- Up to 48 samples per run in 25-60 minutes
- Integrated UV decontamination



DNA was isolated from HTC116 cells grown in Ultra-Low Attachment (ULA) plates (Corning) or Matrigel® Matrix using the Maxwell® RSC Tissue DNA Kit.

Promega's qPCR Chemistries

	Dye-based qPCR	Probe-based qPCR
Genomic DNA / cDNA	GoTaq® qPCR Master Mix	GoTaq® Probe qPCR Master Mix
RNA	GoTaq® 1-Step RT-qPCR	GoTaq® Probe 1-Step RT-qPCR
	GoTaq® 2-Step RT-qPCR	GoTaq® Probe2-Step RT-qPCR

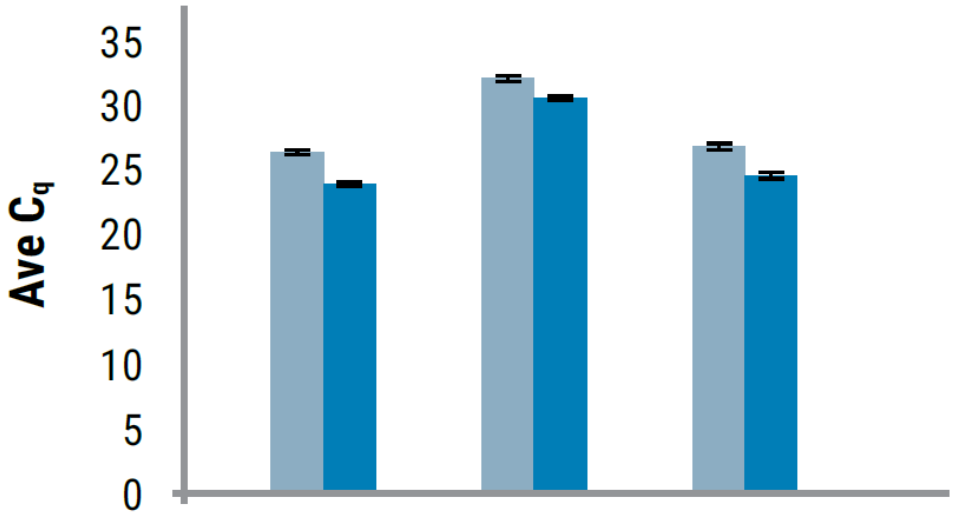


- Low chances of cross contamination
- Faster results
- No need to store the cDNA

- Optimized performance of both RT and PCR steps
- cDNA available for other procedure
- Many targets per sample

GoTaq® Probe	GoTaq® Enviro	GoTaq® Endure
Broad spectrum of applications	Specifically for environmental samples	Specially developed for very high inhibitor concentrations
		
Compatible with variety of samples.	Tested samples like water, soil and biological material	Tested for blood, bacteria, viruses, feces, soil, plants and food samples
Tested for inhibitors, but only up to a certain concentration (e.g. up to 50 µM hematin, see Endure: 500 µM hematin)	Tested for inhibitors such as humic and tannic acid.	Tested for inhibitors such as EDTA, EtOH, Humaic acid, Hematin (500 µM), Heparin, Sodium Citrate but not tested for tannic acid
For general use rather than extreme conditions		Fewer reaction failures and optimizations → Saves time and costs
Multiplexing capability ✓	Multiplexing capability ✓	Multiplexing capability ✓
Probe-based ✓	Probe-based ✓	Probe-based ✓
Fast-Cycling ✓	Fast-Cycling ✓	Fast-Cycling ✓

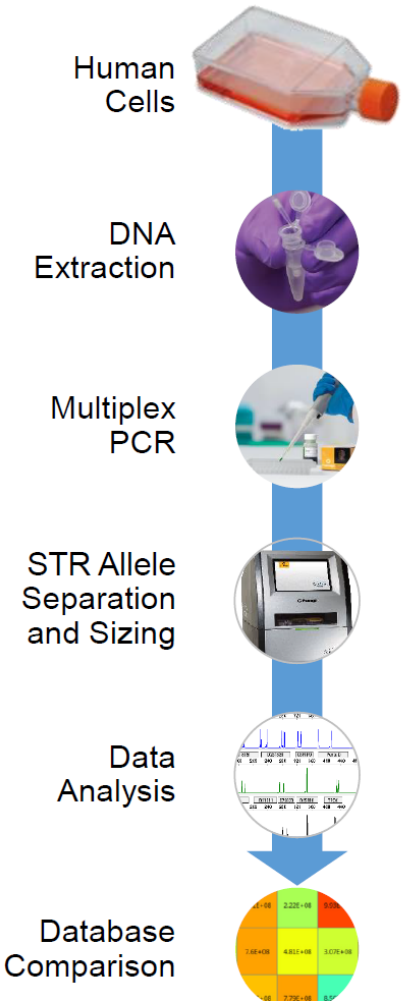
GoTaq™ qPCR Family



	GNB2L1	HPRT1	B2M
3000 HCT116 Cells	25.82	31.43	26.25
10000 HCT116 Cells	23.56	30.10	24.20

HCT116 cells were seeded at two different densities on GravityPLUS™ hanging drop 96-well plates to form spheroids

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

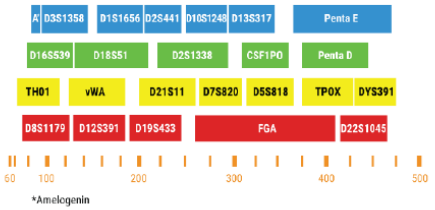
Both Systems are compatible with the Spectrum Compact CE System and with the Applied Biosystems® Genetic Analyzers.

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

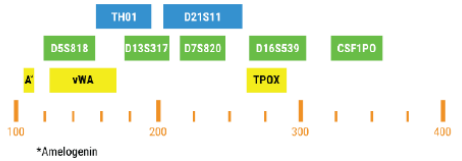
Recommended for patient-derived cell lines and meets the ASN-0002-2011 Guidelines

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



Questions?

For additional questions please contact:
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Let's connect!

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