Bioluminescence in Action: Monitoring Cell Health, Metabolism, and Protein Dynamics

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Our Products Support

Government and Academic Research Laboratories

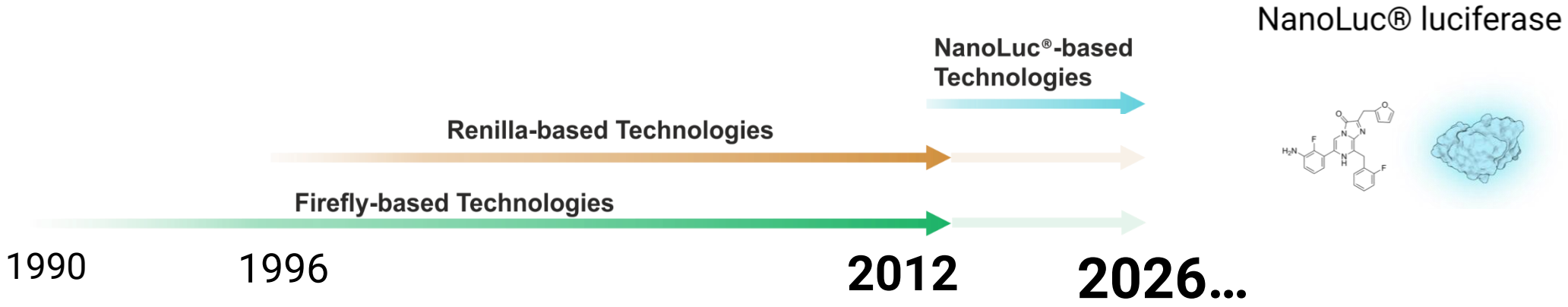
Forensic and Paternity Laboratories

Pharmaceutical and Biotechnology Industries

Clinical and Molecular Diagnostics Laboratories

Food and Water Safety Testing Facilities

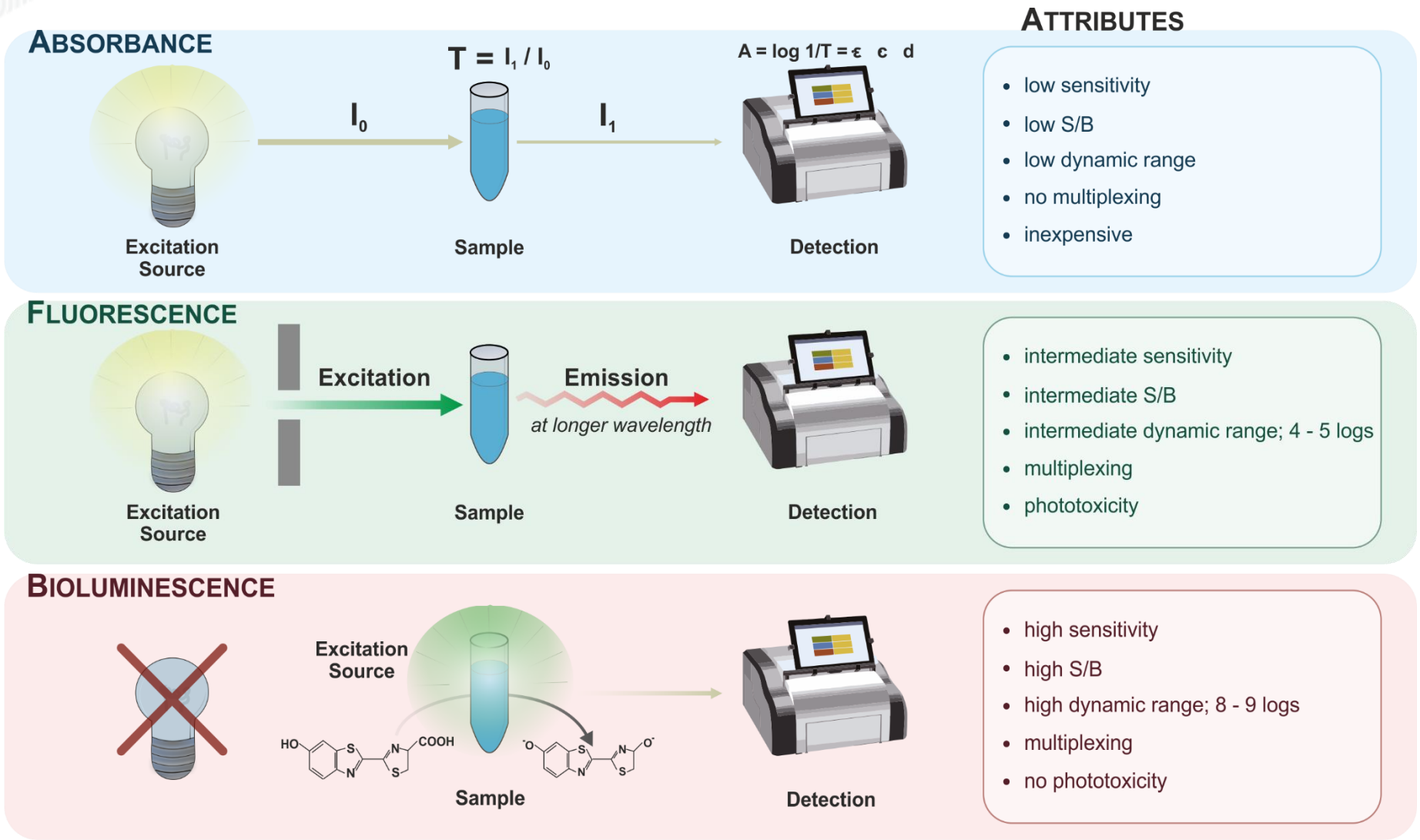
Decades of Experience with Bioluminescence



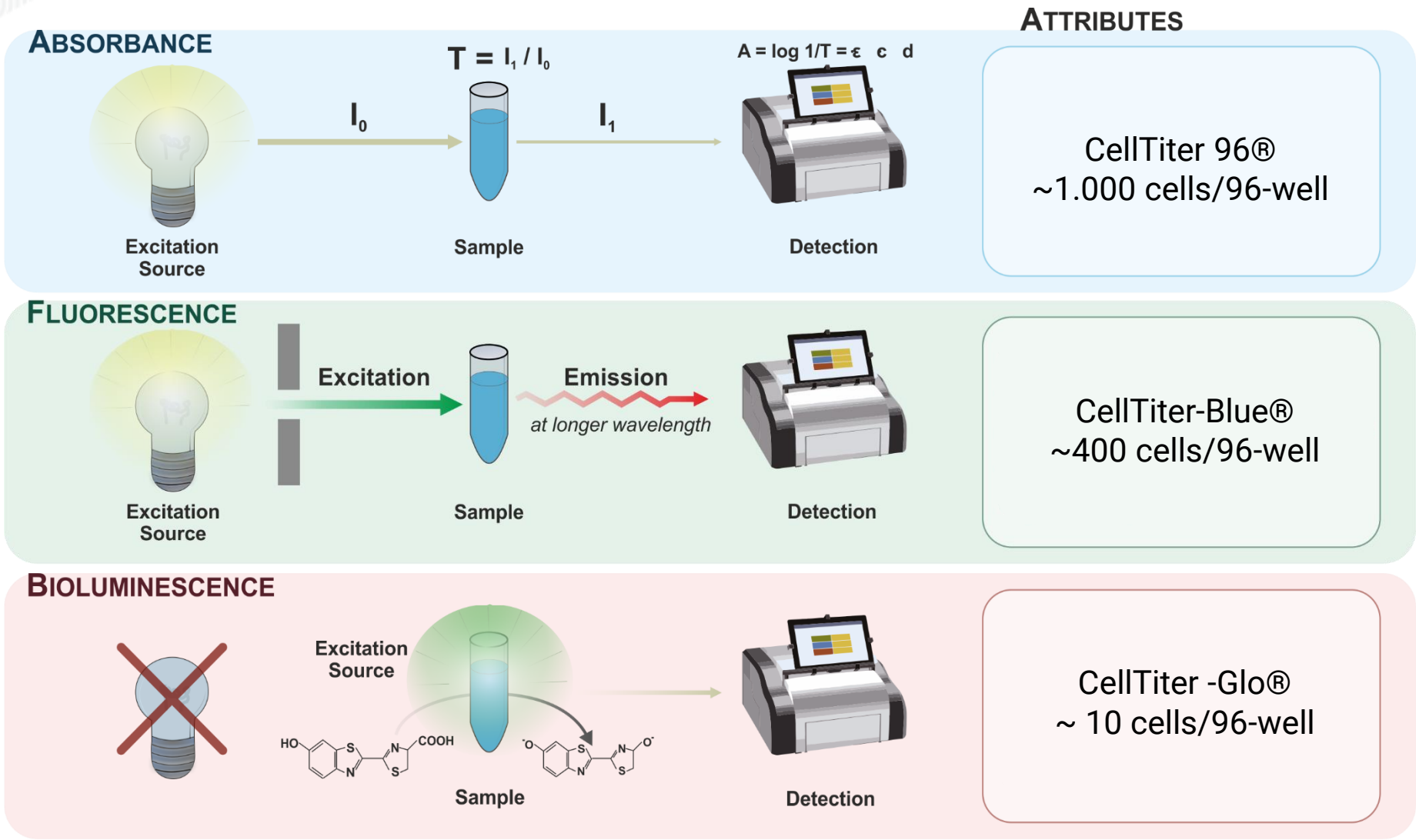
- Reporter Gene Assays
- GloSensor™ (cAMP, Protease Assays)
- GloResponse™ (Signaling Pathways)
- Rapid Response™ (Signaling Pathways)
- Cell-Health Assays
- Bioassays (ADCC, PDL1..)

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

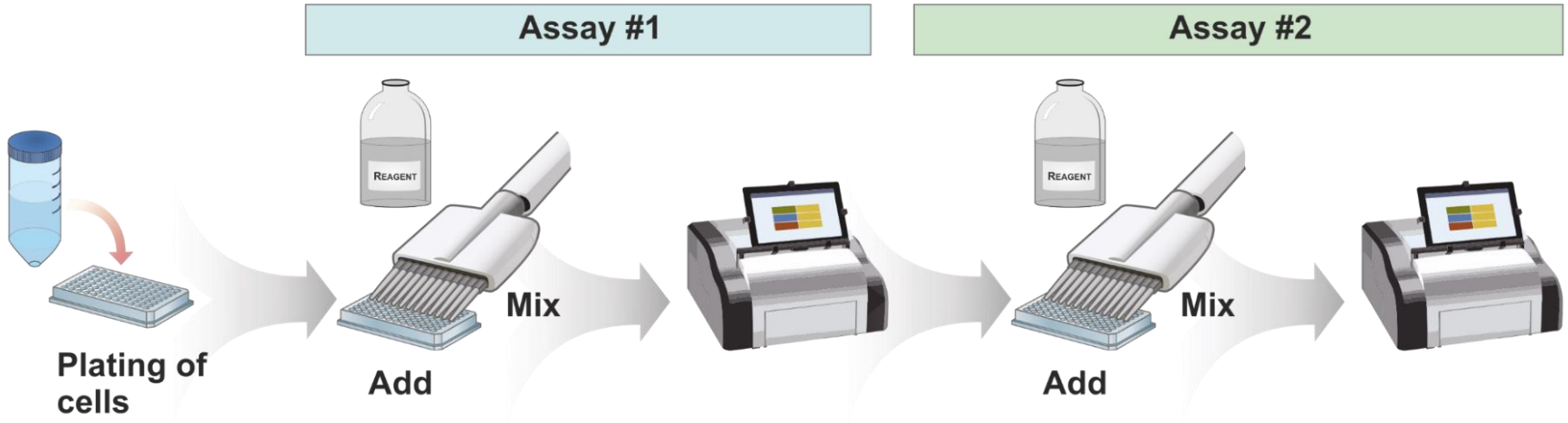
Assays Detection Systems



Assays Detection Systems



Assay Multiplexing



Prerequisites

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

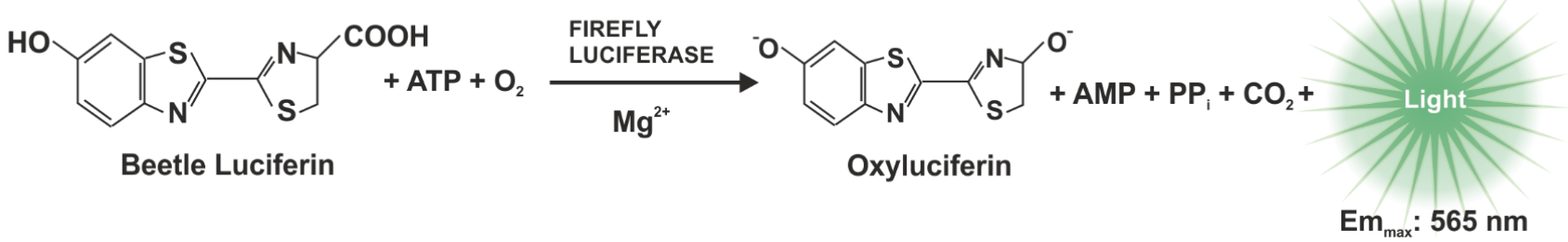
Benefits

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

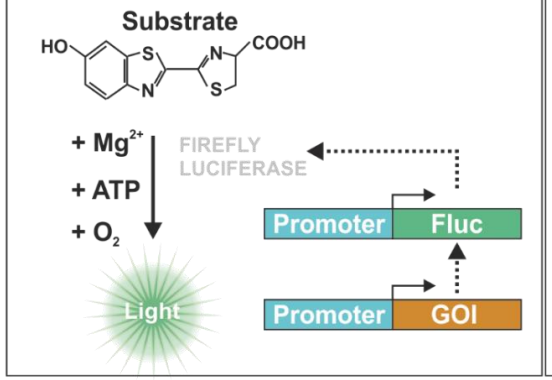
Firefly Luciferase is Key to Many Promega Cell-Based Assays

Ultra-Glo™ rLuciferase

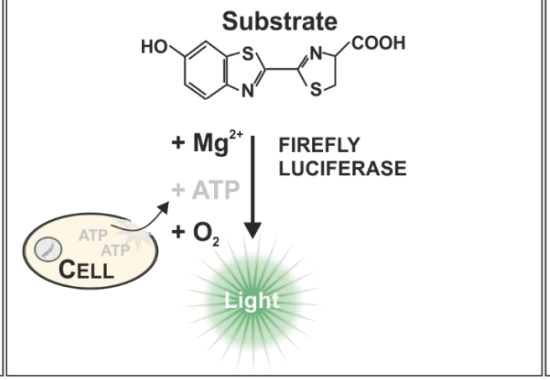
- Greater thermal stability
- Greater pH tolerance
- Greater detergent/reducing agent tolerance
- Reduced compound interference



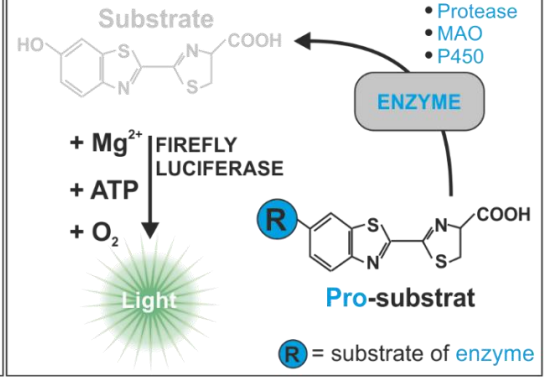
Type I — Luciferase expression



Type II — ATP content

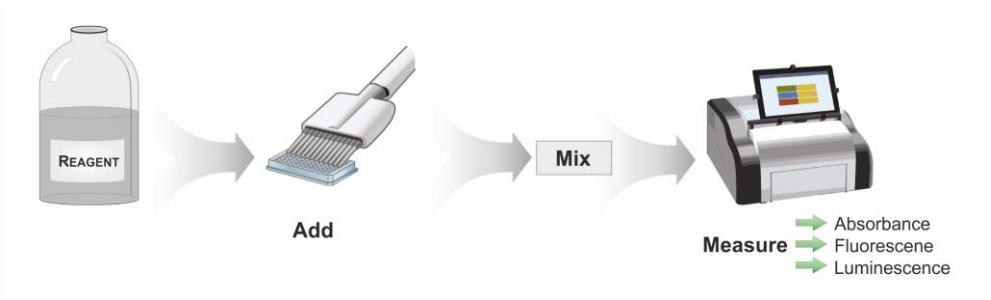


Type III — Substrate release



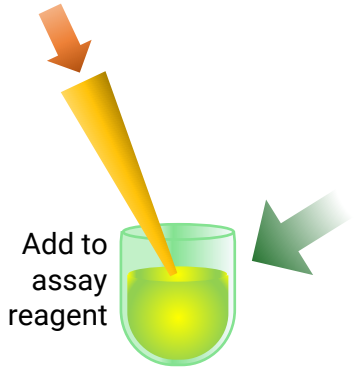
grey = limiting factor

Promega's Cell-based Assay Portfolio



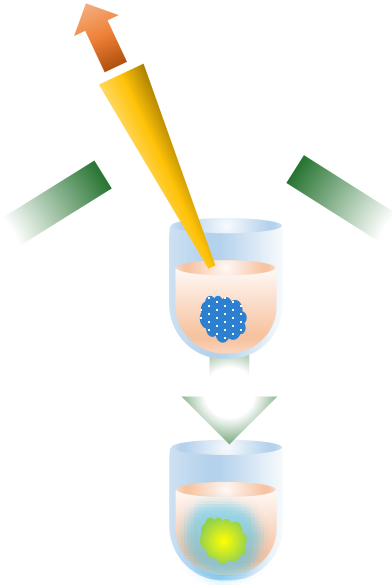
Benefits

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



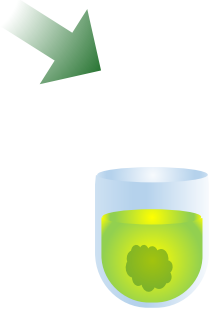
Media Sampling
Non-Lytic Assays

LDH-Glo™ Cytotoxicity Assay



Live Cell Assays
Non-Lytic Assays

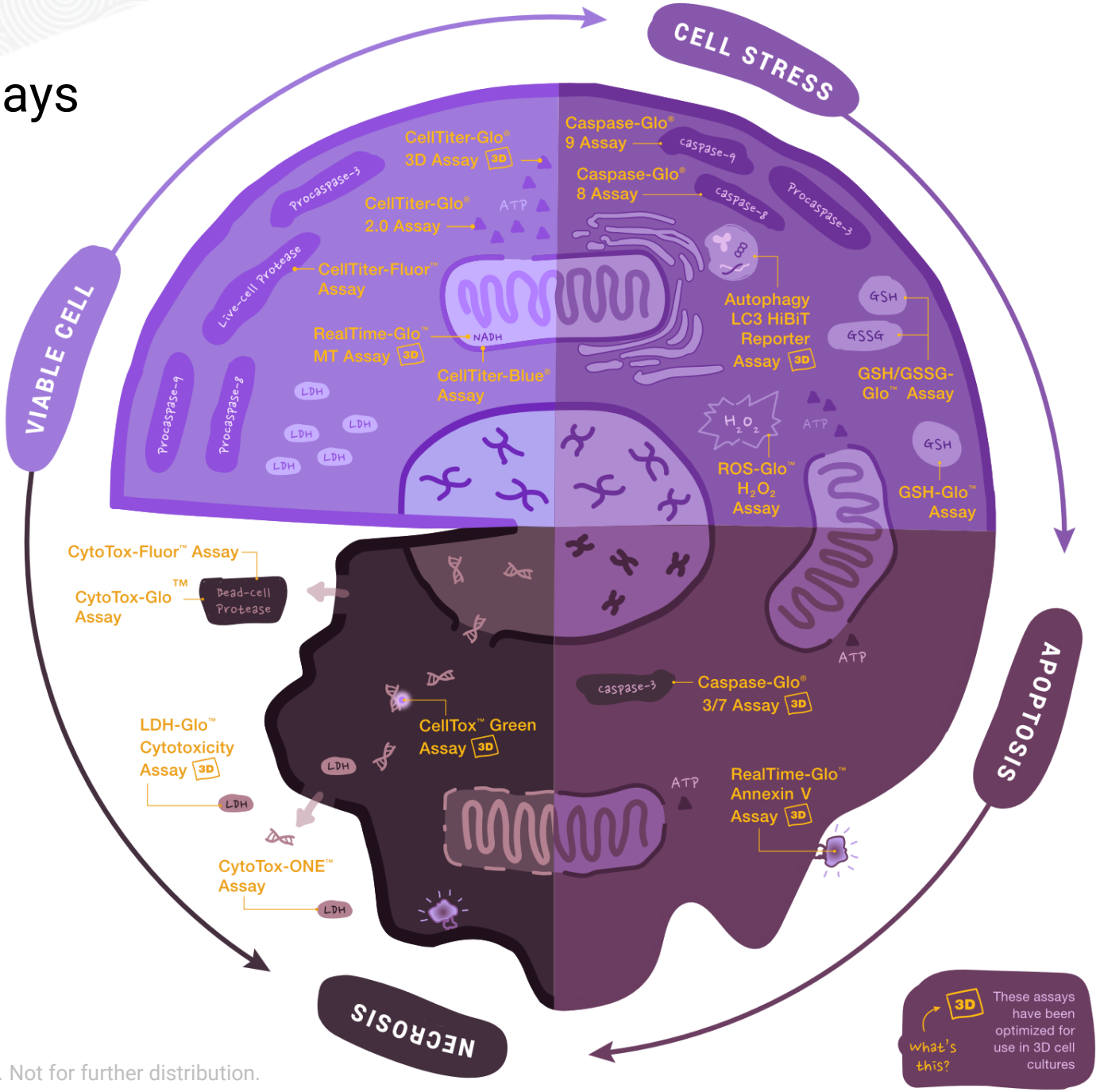
RealTime-Glo™ MT Cell Viability Assay



End-Point
Lytic Assays

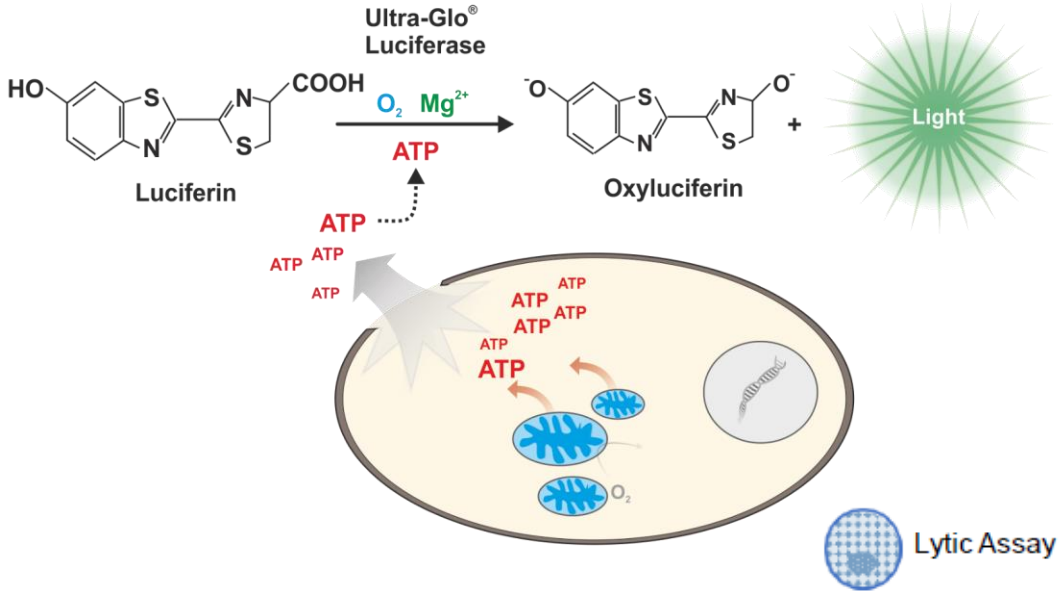
CellTiter-Glo® 2.0 Cell Viability Assay

Cell Health Assays

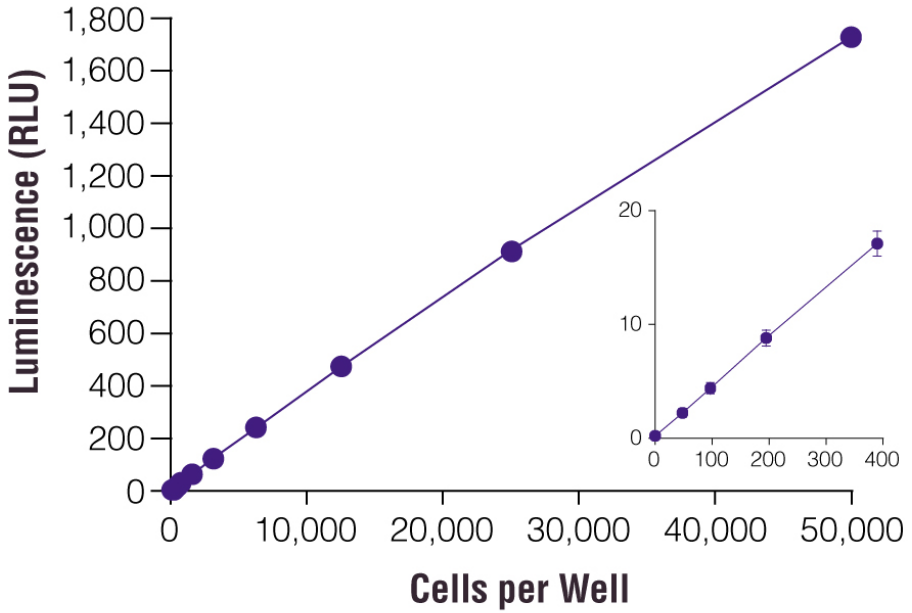


Cell Health Assays: Cell Viability

CellTiter-Glo® 2.0 Assay

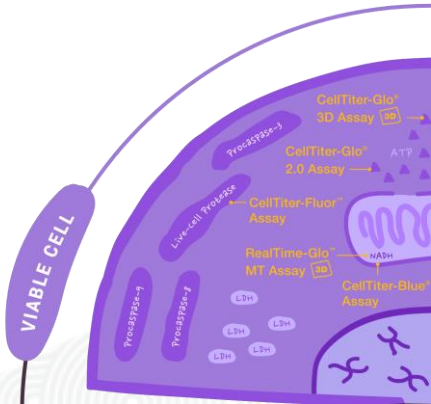


CellTiter-Glo® 2.0 Assay



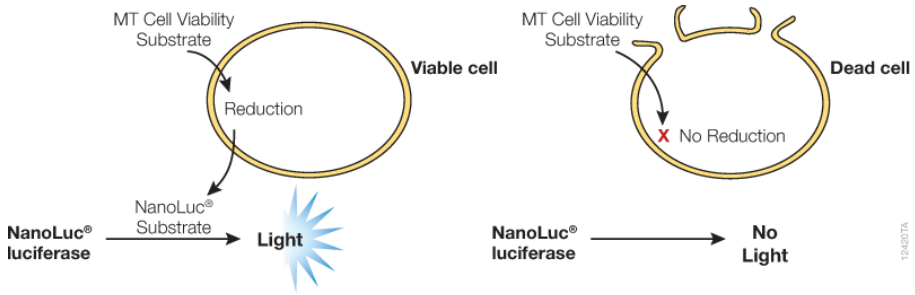
- Linear range: 10 – 50000 cells
- Signal stability: Half life > 5 (3)* hours
- Robust: High Z'-factor
- No extensive incubation required

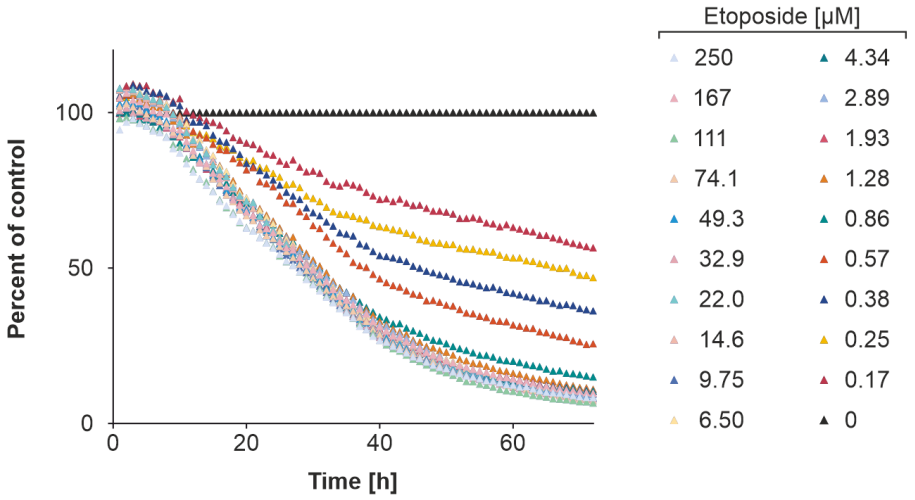
* CellTiter-Glo® 2.0



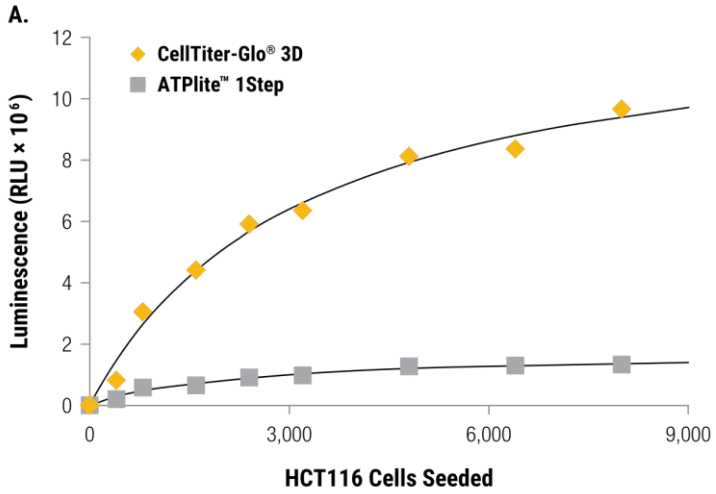
Cell Health Assays: Cell Viability

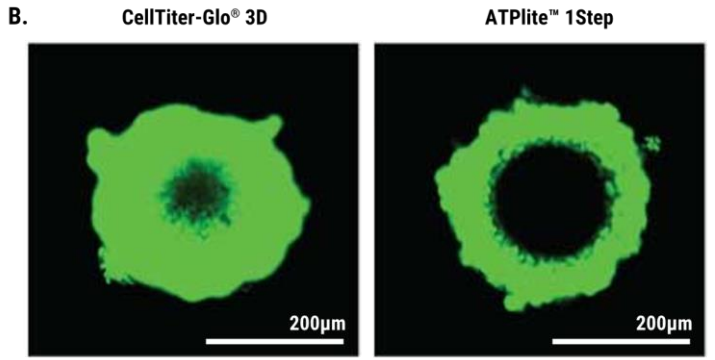
RealTime-Glo™ MT Cell Viability Assay

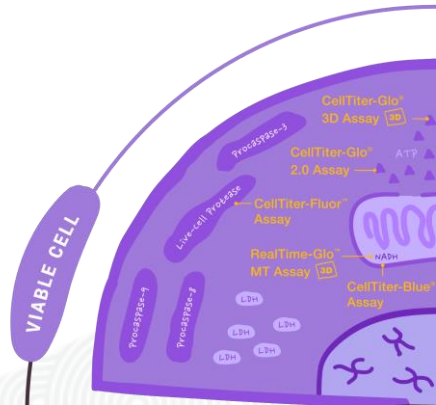




CellTiter-Glo® 3D Cell Viability Assay

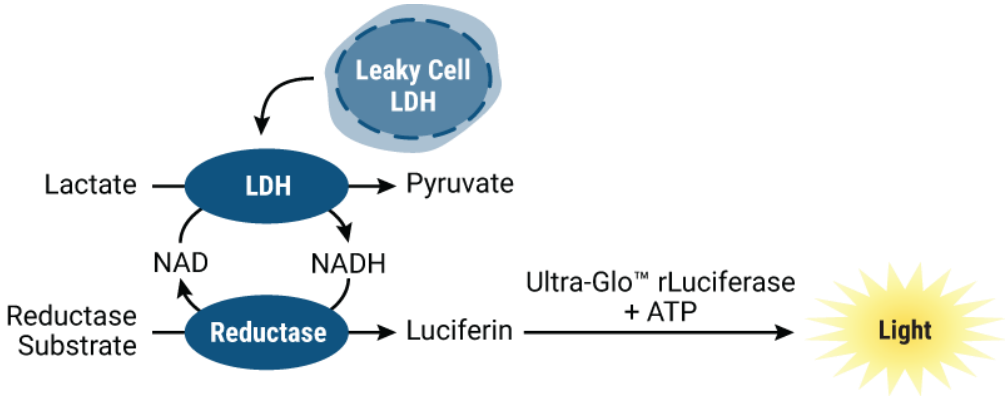




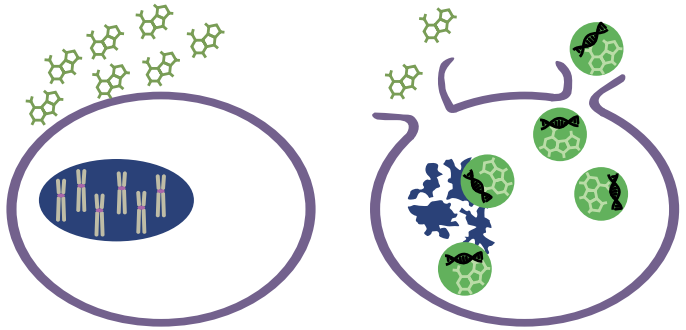


Cell Health Assays: Cell Toxicity

LDH-Glo™ Cytotoxicity Assay

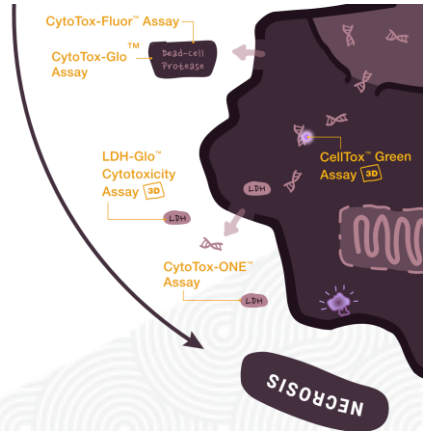


CellTox™ Green Cytotoxicity Assay

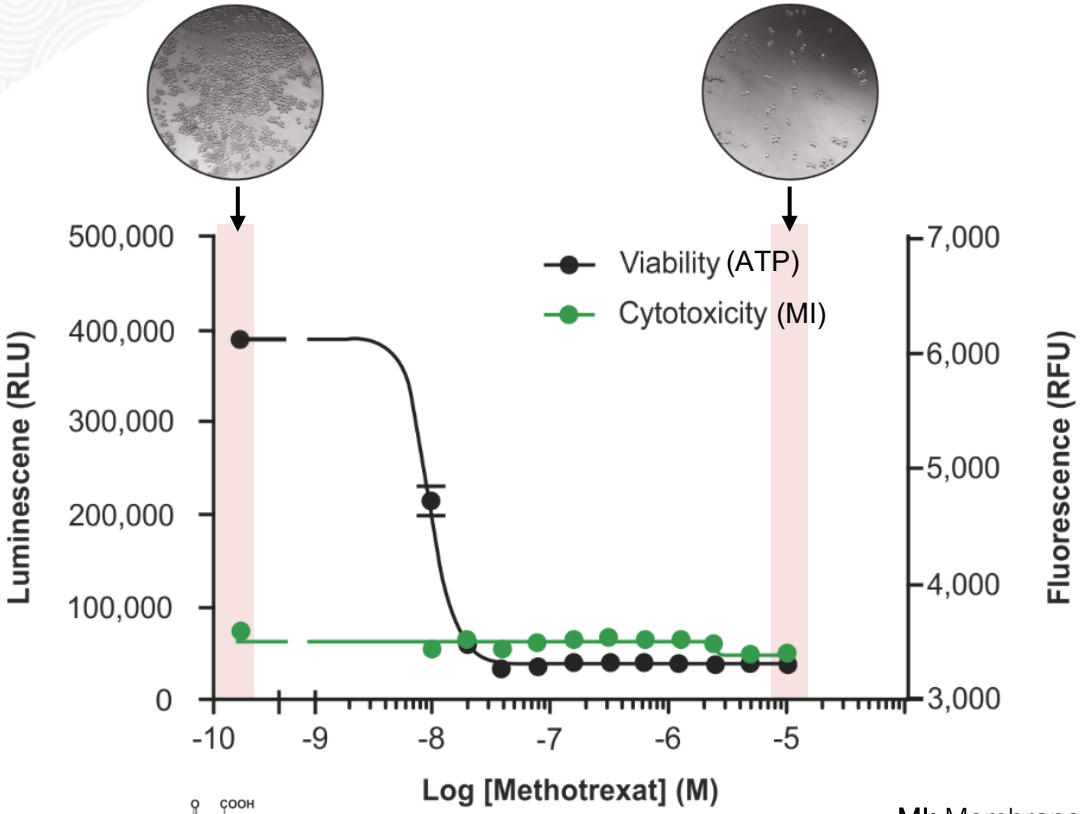


Low Fluorescence Viable Cells

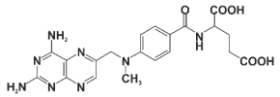
High Fluorescence Nonviable Cells



Cell Health Assays: Cell Toxicity

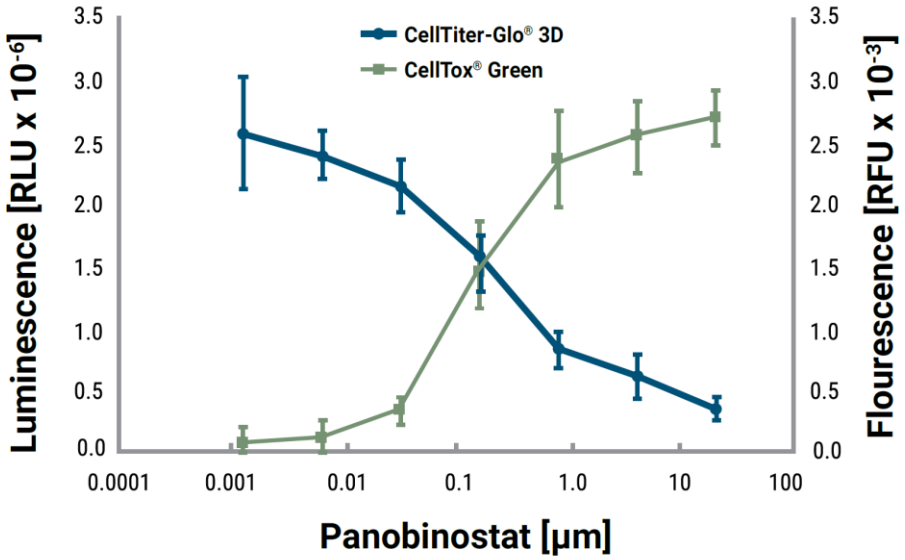


MI: Membrane Integrity

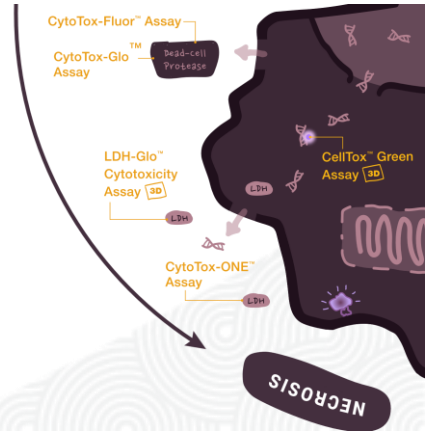


DHFR inhibitor

Nucleotide biosynthesis → Cell division

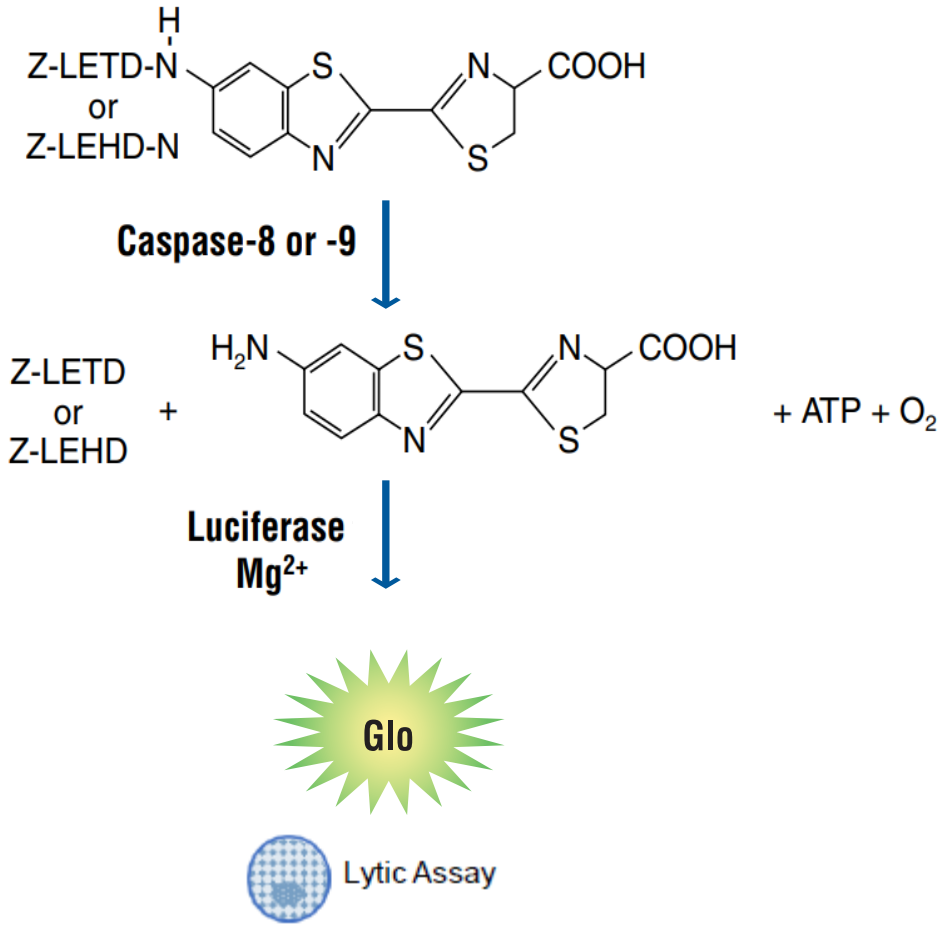


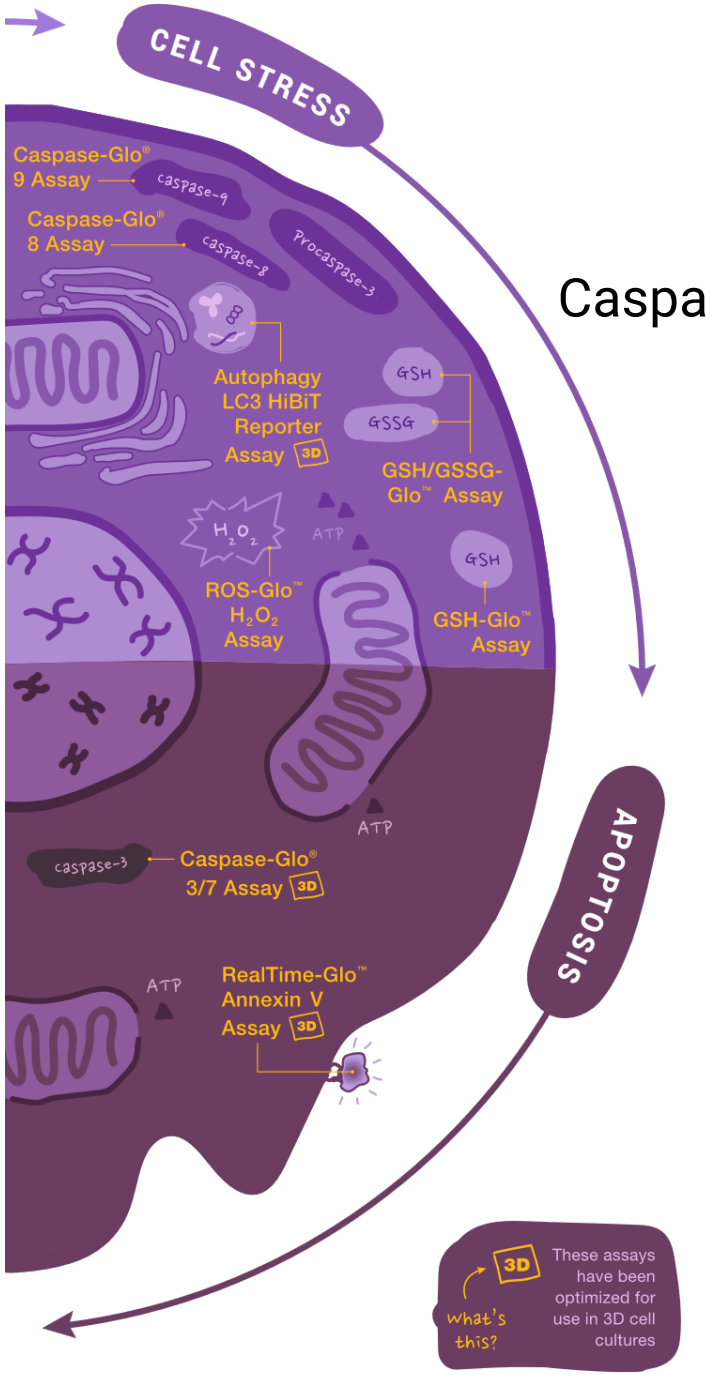
HCT116 colon cancer spheroids were grown for 4 days in a 96-well hanging drop plate



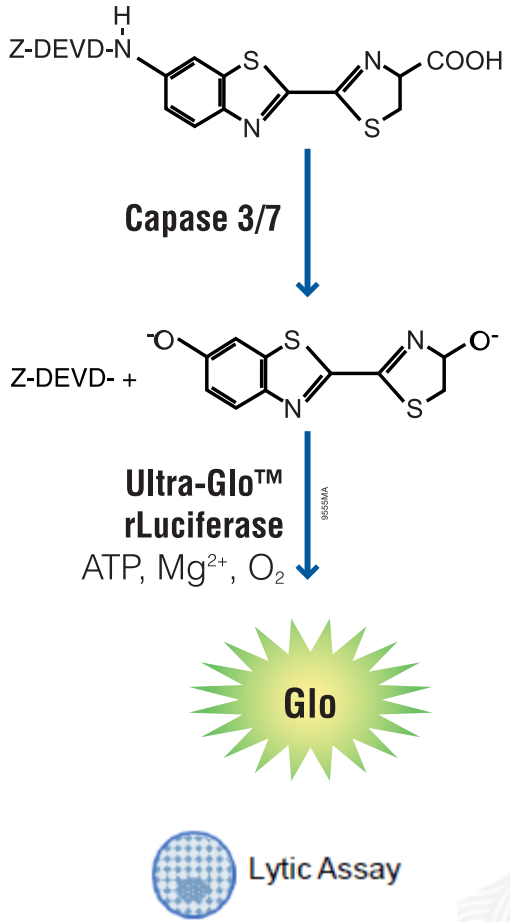
Cell Health Assays: Apoptosis

Caspase-Glo® 8 or 9 Assay Systems

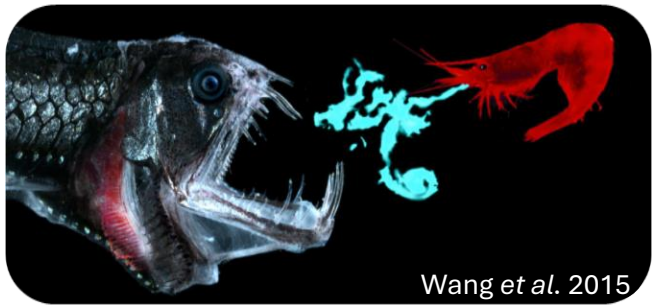




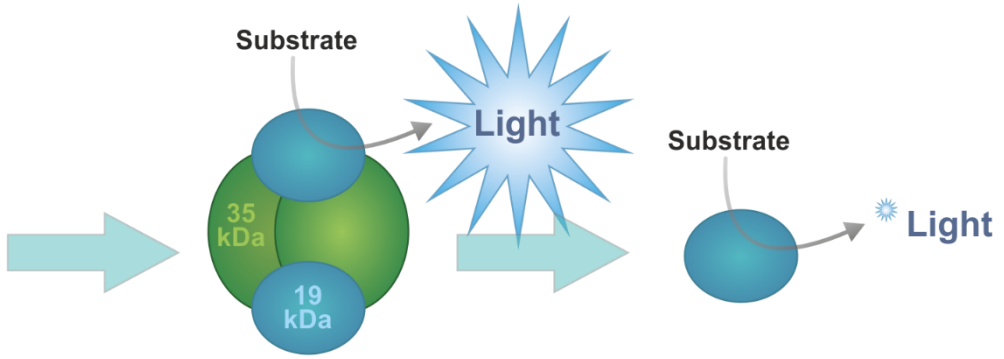
Caspase-Glo® 3/7 Assay System



NanoLuc® Luciferase: A Bright and Small Reporter



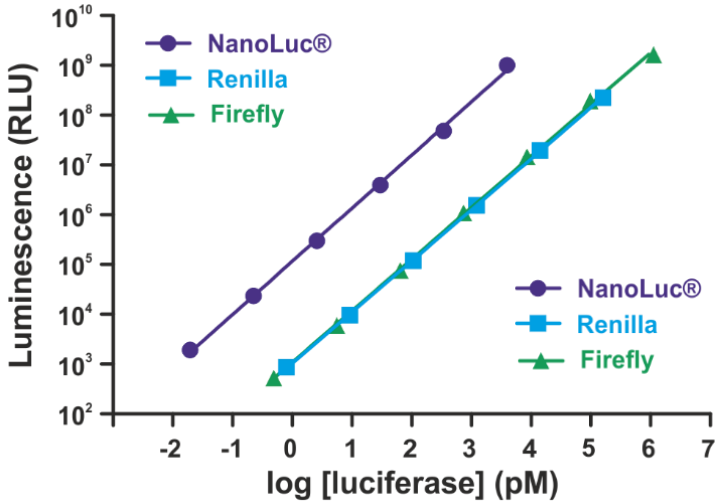
Oplophorus gracilirostris



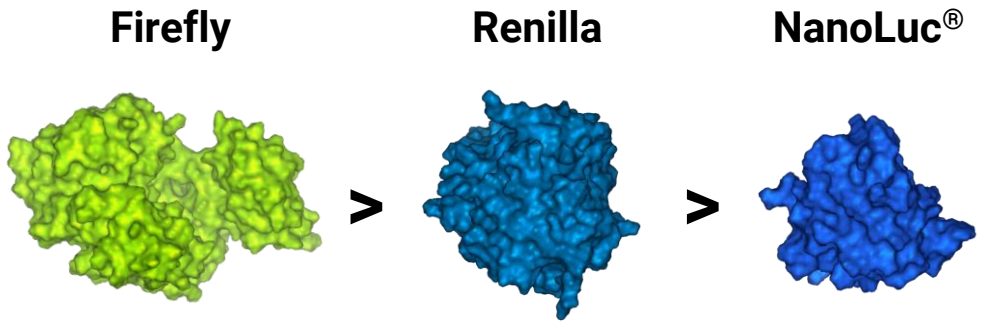
- *Oplophorus* luciferase 106 kDa
- 7x brighter than Rluc
- Glow luminescence
- Shimomura *et al.* 1978

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

Bright, Brighter, NanoLuc®

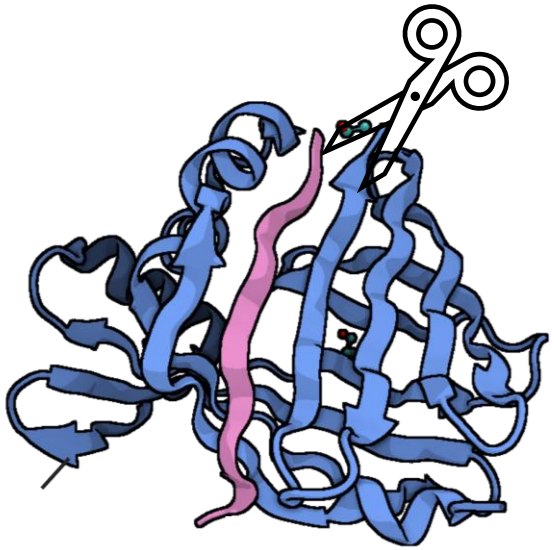


Small, Smaller, NanoLuc®



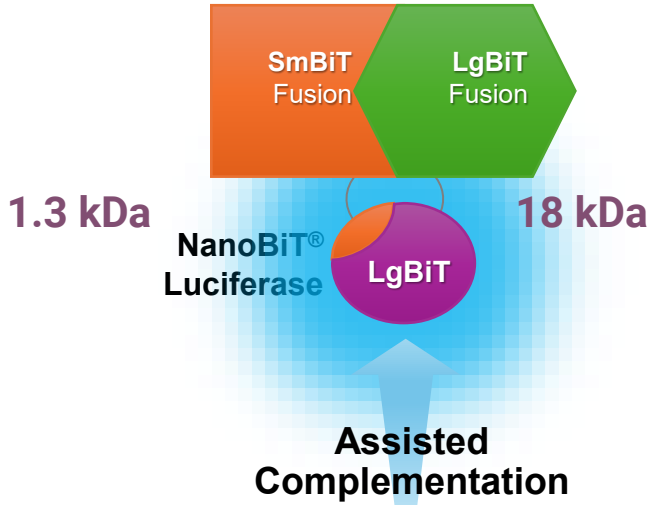
60.6 kDa | 36 kDa | 19.1 kDa

NanoLuc® Binary Technology (NanoBiT®)



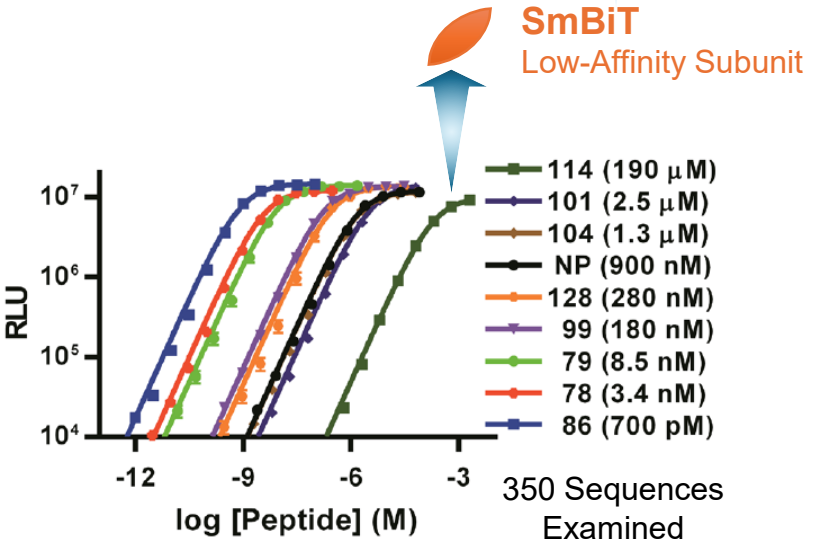
NanoLuc®

Protein:Protein Interactions



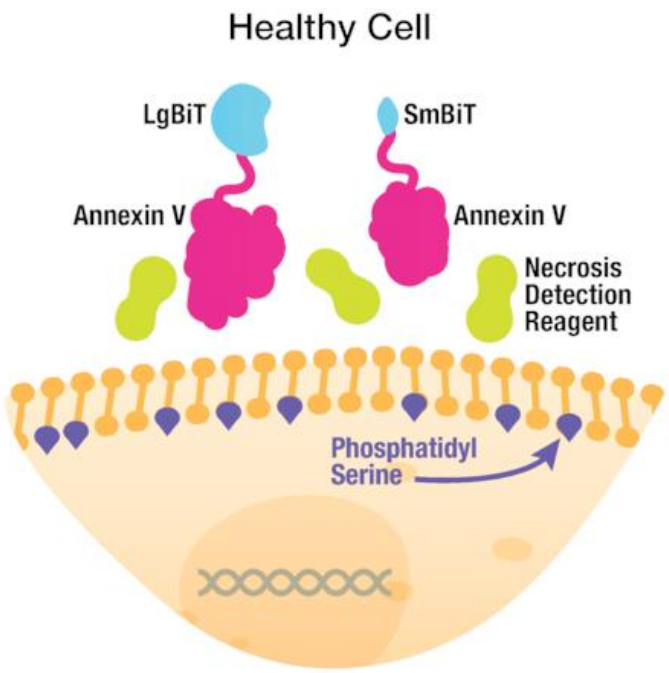
Small tag Size
minimal influence on fusion partner

Bright signal upon complementation enables low expression levels

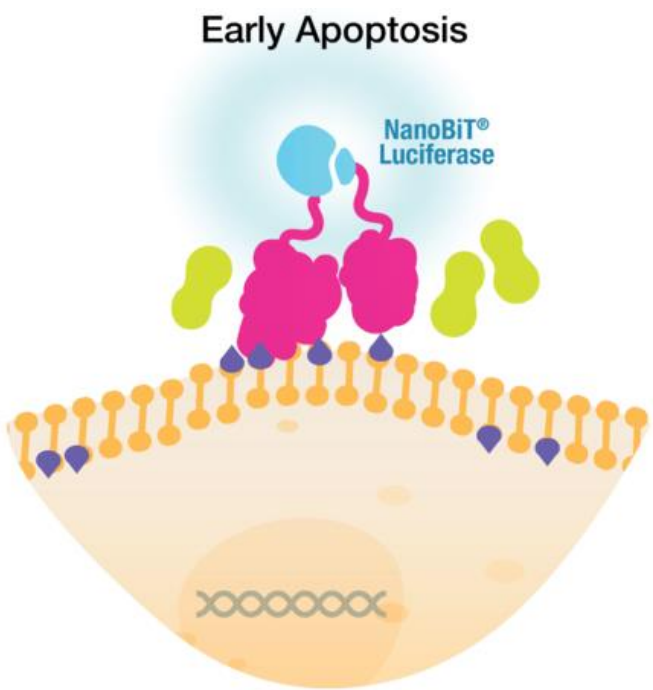


350 Sequences Examined

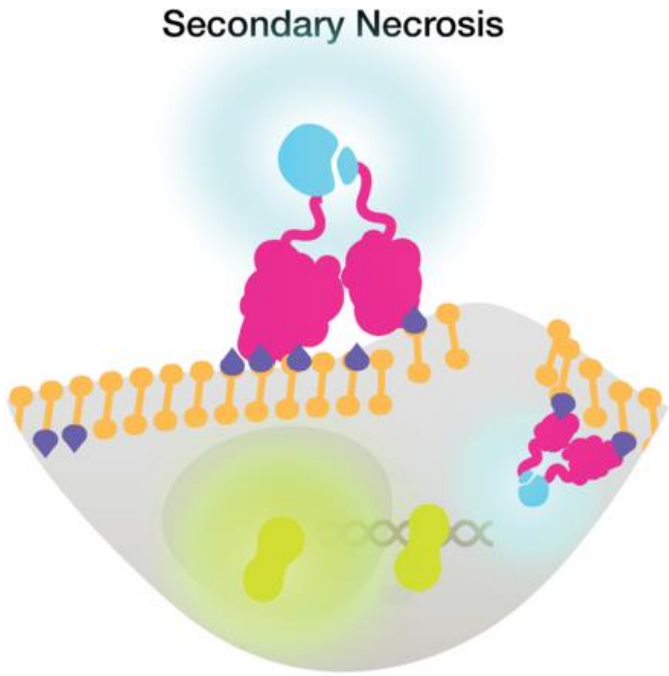
RealTime-Glo® Annexin V Apoptosis and Necrosis Assay



PS confined to inner leaflet
Cell membrane intact
Luminescence (RLU) negative
Fluorescence (RFU) negative

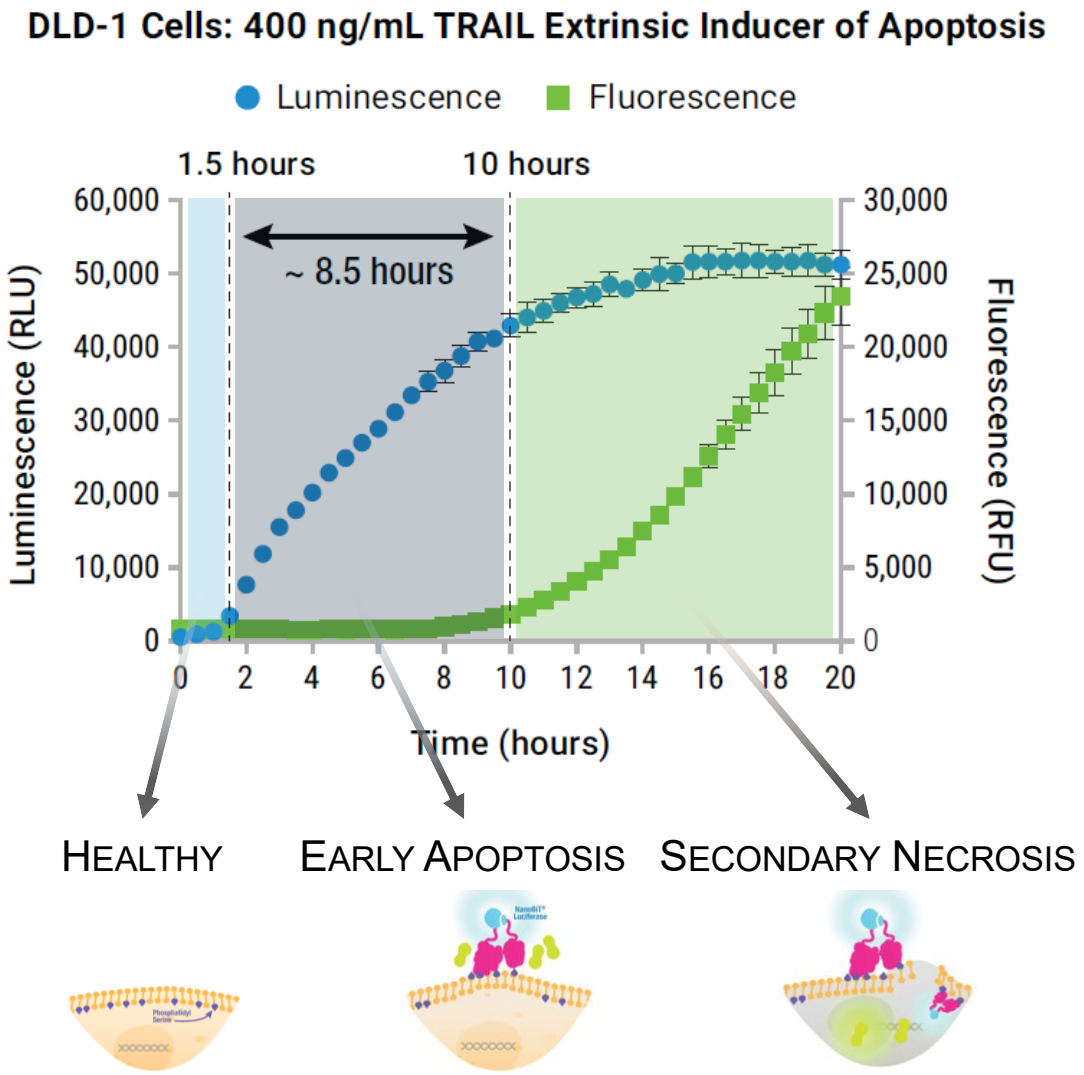


PS translocation to outer leaflet
Cell membrane intact
Luminescence (RLU) POSITIVE
Fluorescence (RFU) negative

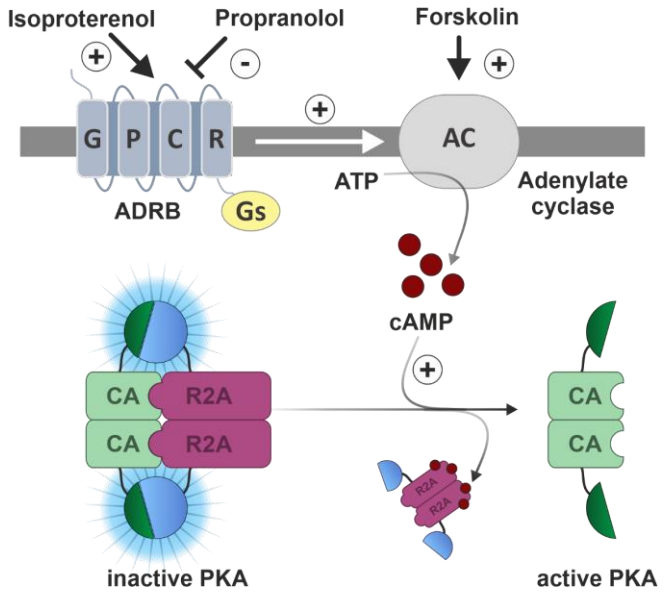


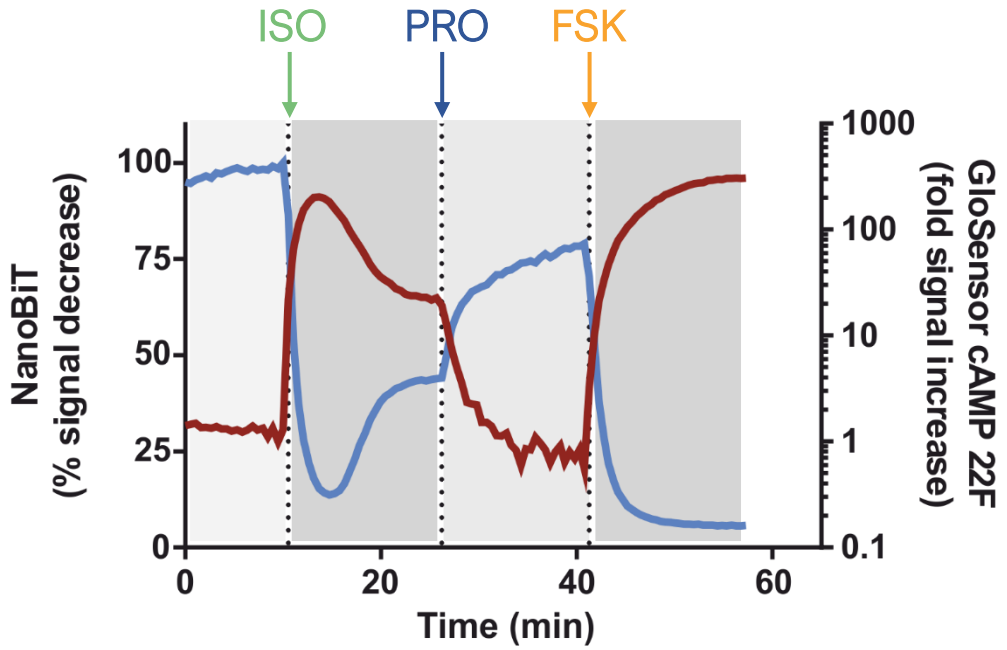
PS on inner and outer leaflet
Cell membrane compromised
Luminescence (RLU) POSITIVE
Fluorescence (RFU) POSITIVE

What does the data look like?



Validation of NanoBiT® PPI

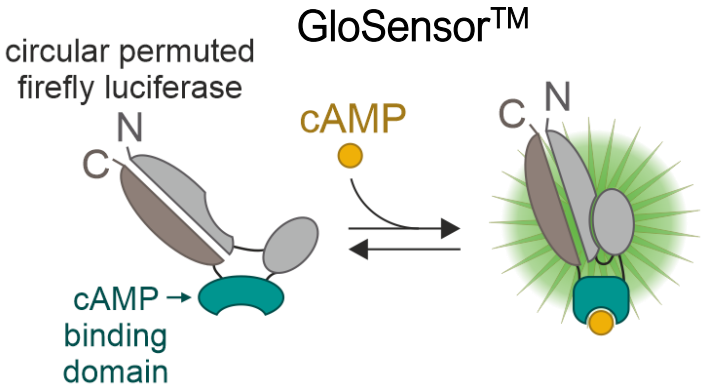




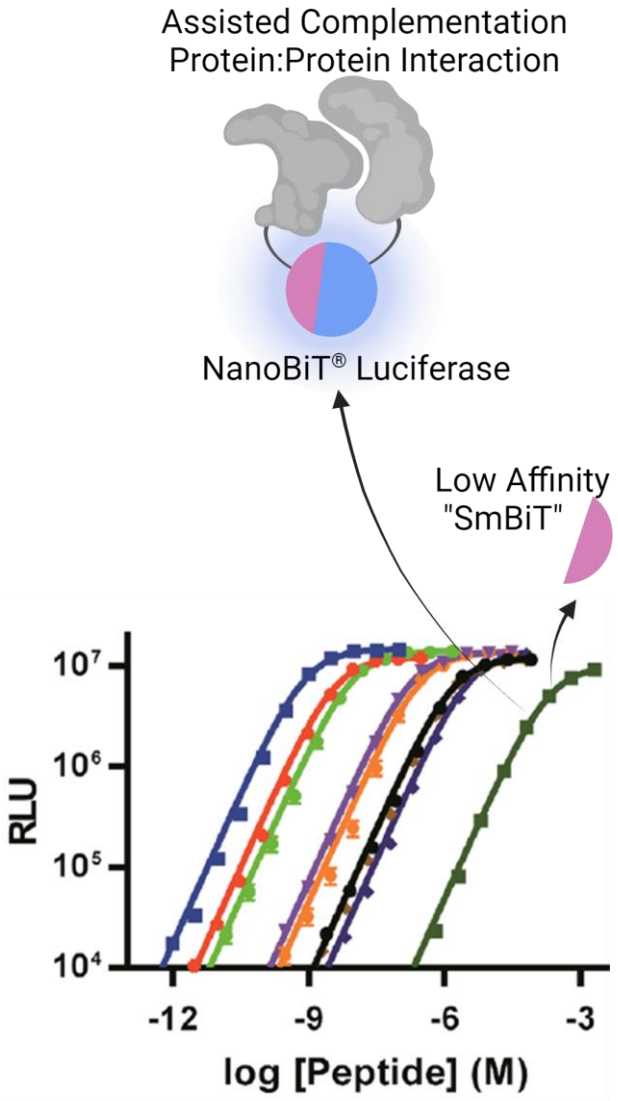
Isoproterenol (ISO)
ADRB agonist (cAMP ↑)

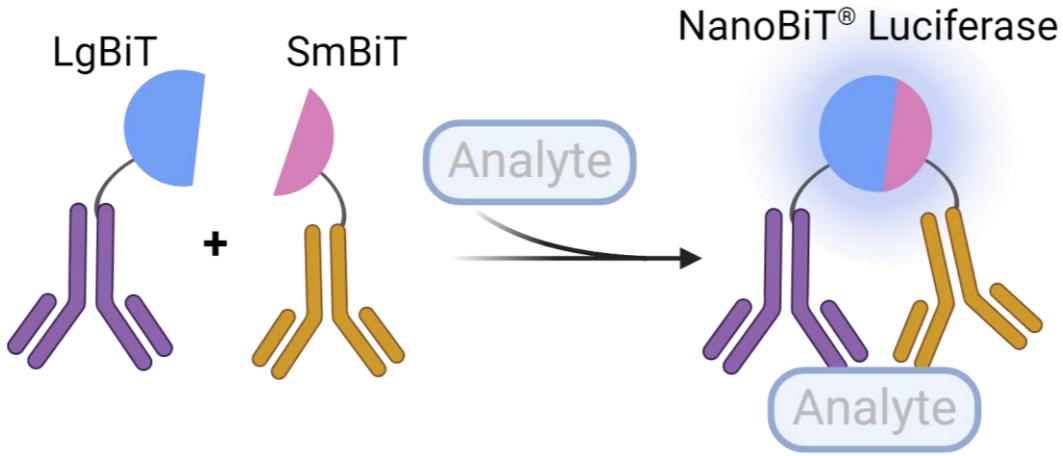
Propranolol (PRO)
ADRB antagonist (cAMP ↓)

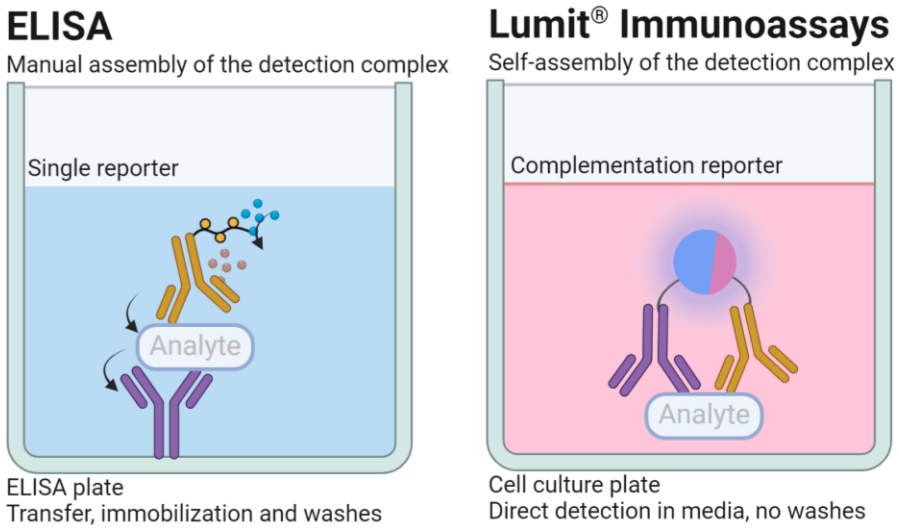
Forskolin (FSK)
activator of adenylate cyclase (cAMP ↑)



Lumit® Immunoassays: Detect Analytes and Molecular Interactions

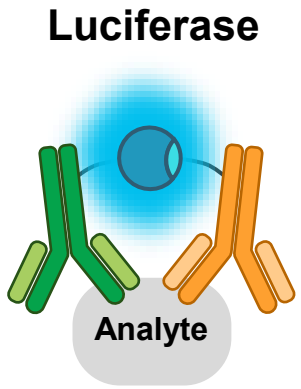






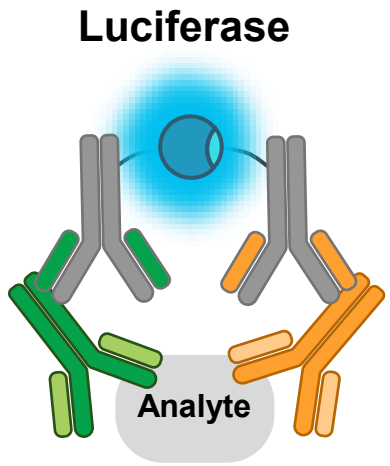
Lumit® Immunoassays: Formats

Direct



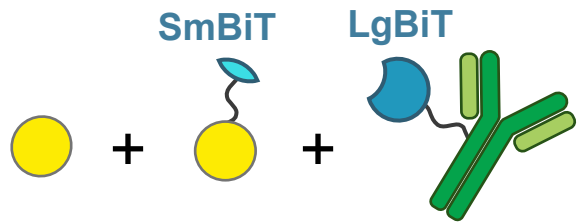
- 1º antibodies labeled w/ NanoBiTs
- Maximizes number of Ab pairs can test for assay development

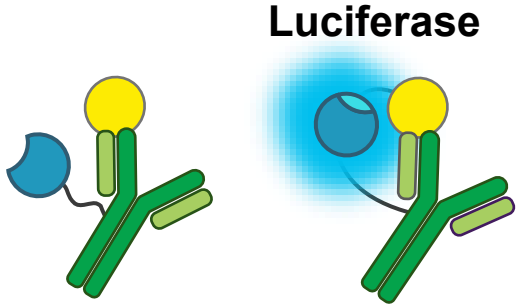
Indirect



- 2º antibodies labeled w/ NanoBiTs
- With unlabeled 1º Abs, enables rapid assay development

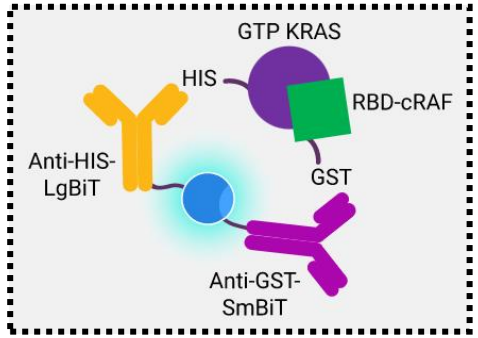
Competitive Binding



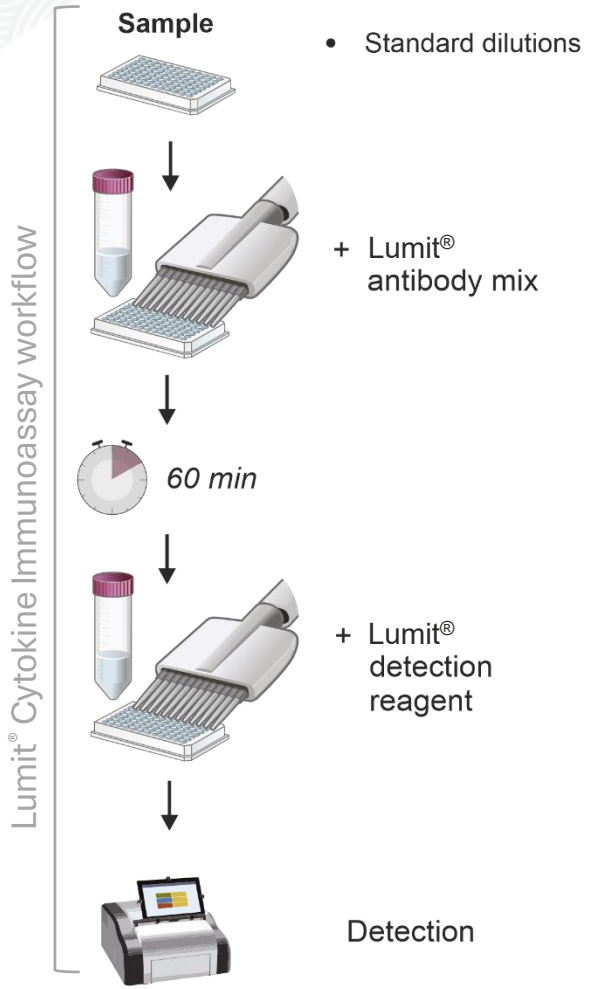


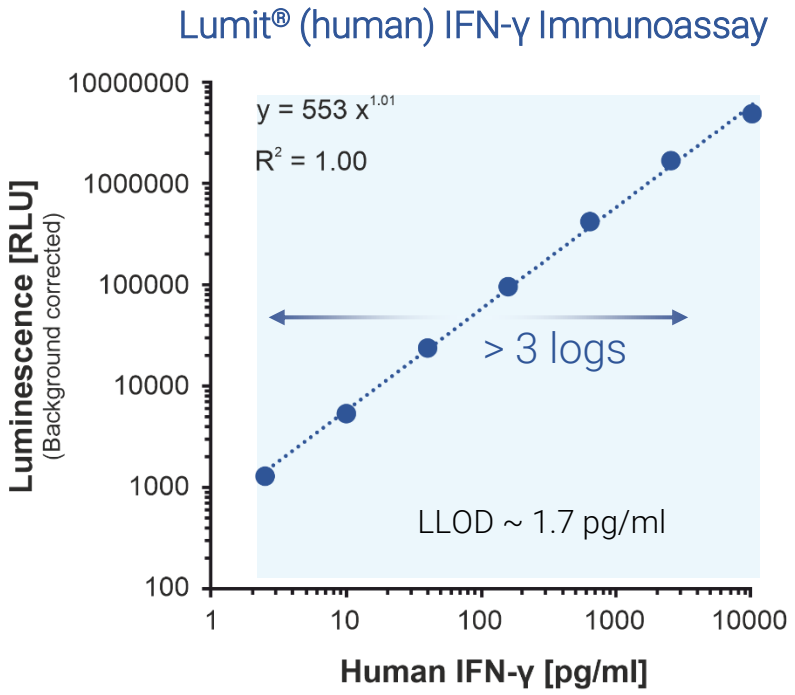
Requires antibody and tracer labeling

…and more!



Lumit® Cytokine Immunoassays

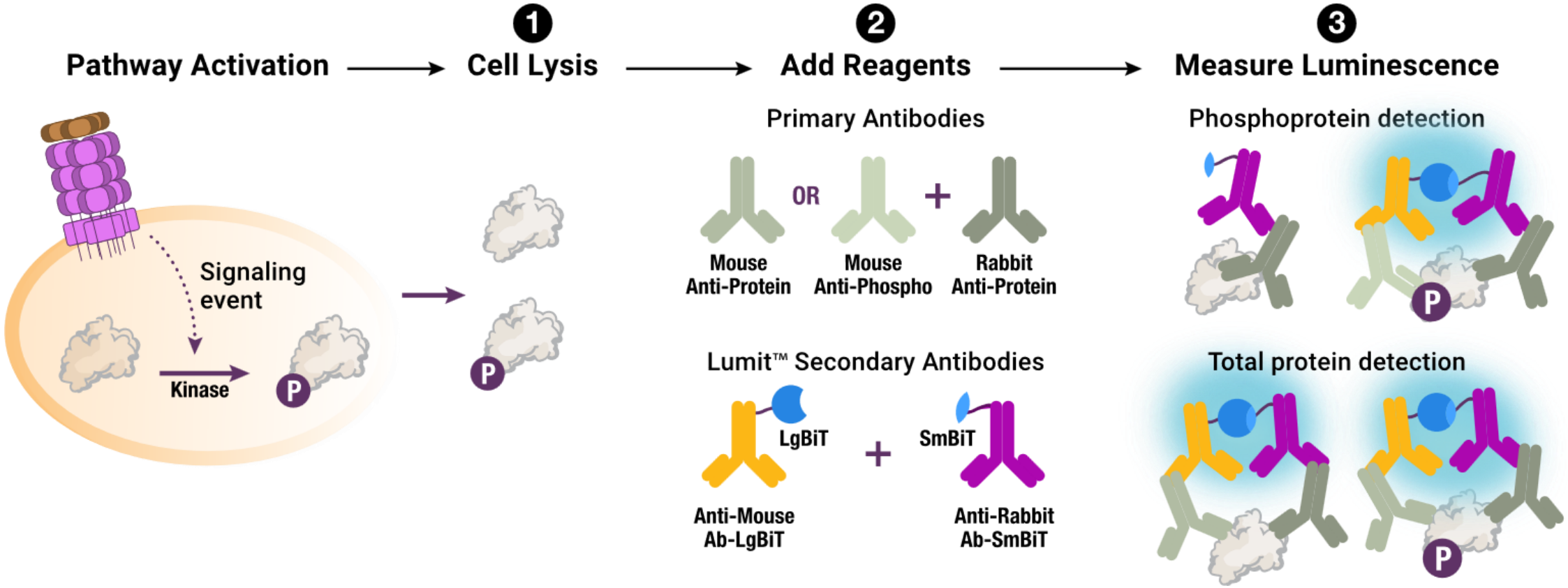




Assay	Dynamic range	LOD
Lumit® IL-2 (Human)	28.2 – 25000 pg/ml	11.2 pg/ml
Lumit® IL-4 (Human)	18.2 – 25000 pg/ml	6.7 pg/ml
Lumit® IL-6 (Human)	18.2 – 25000 pg/ml	7.5 pg/ml
Lumit® IL-10 (Human)	18.2 – 25000 pg/ml	7.4 pg/ml
Lumit® IFN-γ (Human)	7.2 – 10000 pg/ml	1.7 pg/ml
Lumit® TNF-α (Human)	18.2 – 25000 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

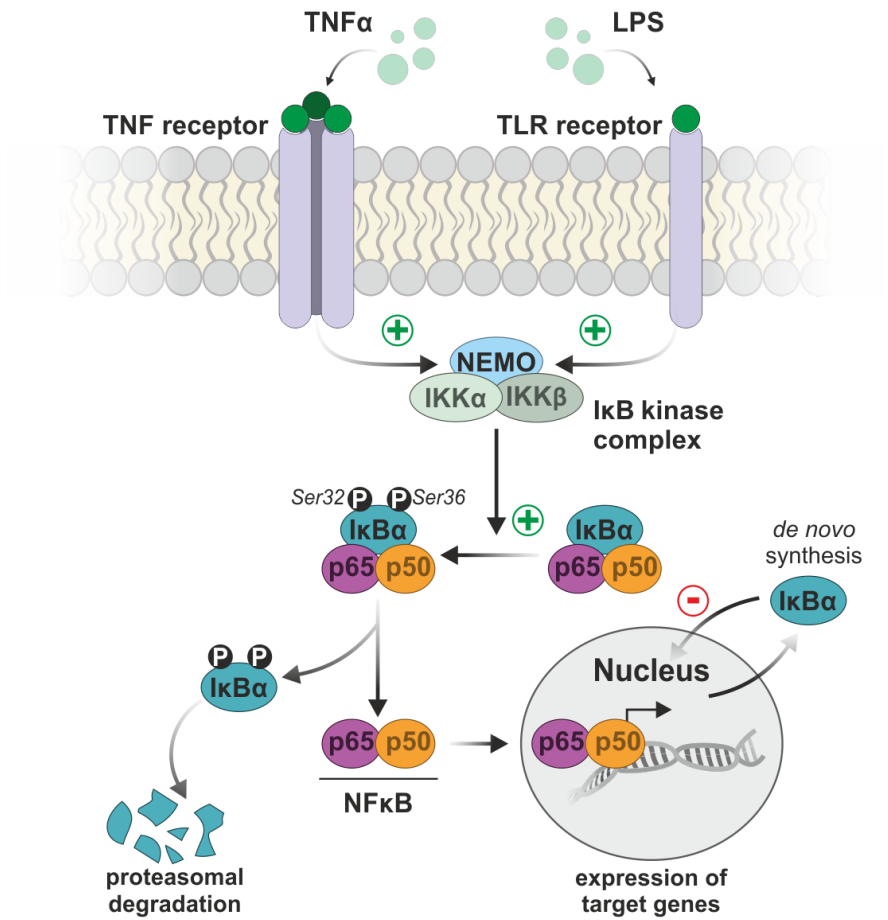
- Good sensitivity and broad dynamic range (~ 3 logs)
- No need for sample dilution
- Flexible detection protocols

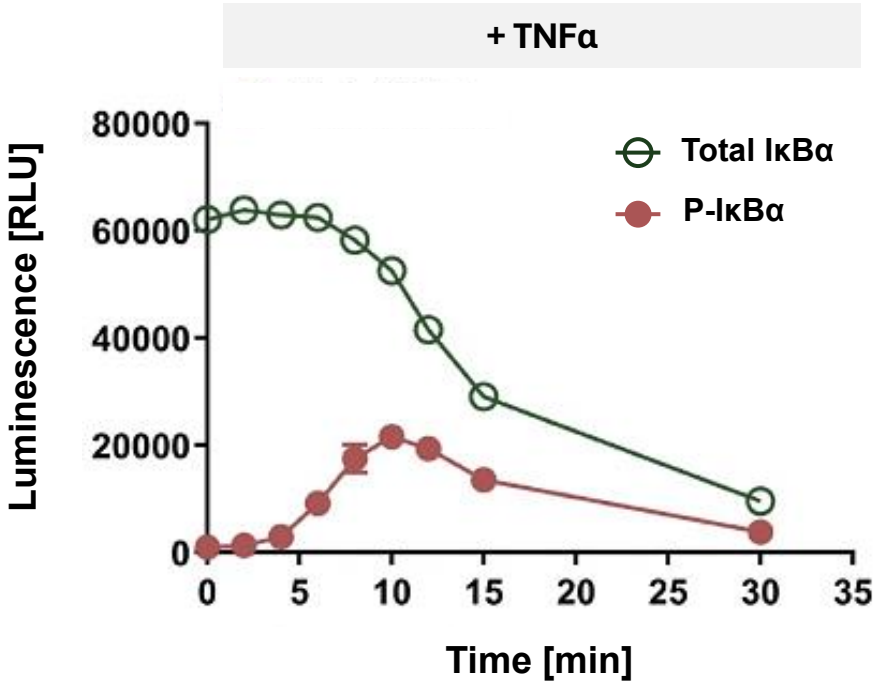
Lumit® Immunoassay Cellular Systems



Cellular Signaling Pathway Analysis

NFκB Signaling Pathway



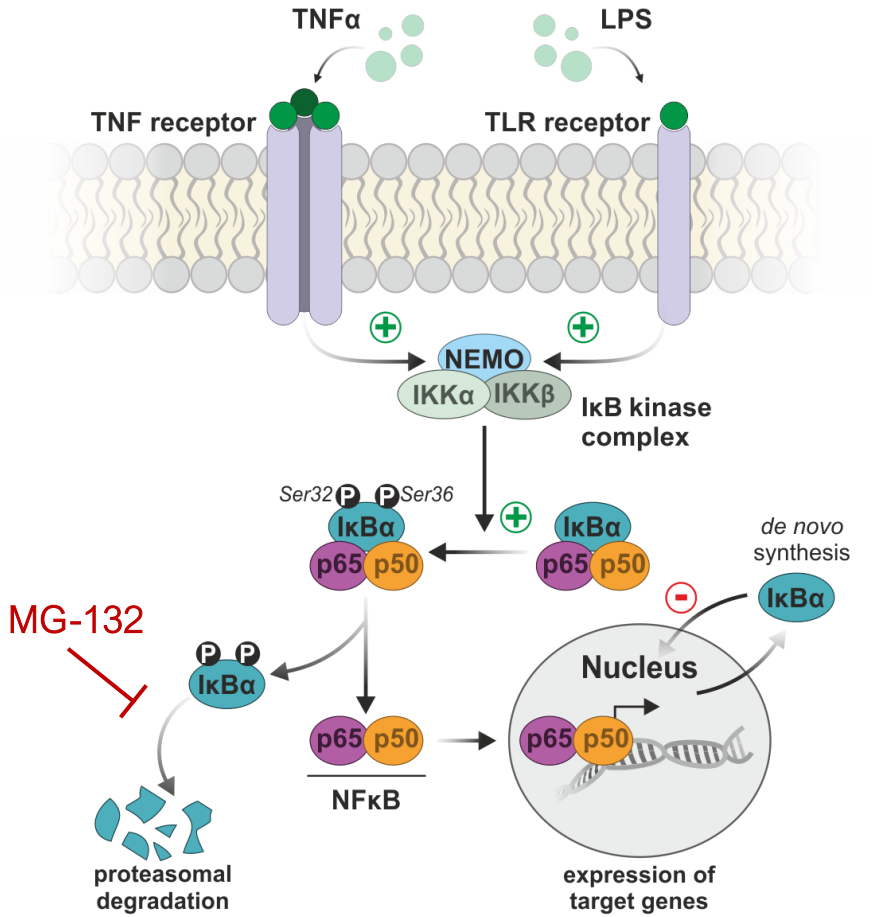


Modified from Hwang, B. *et al.* (2020) Commun Biol. 3:8

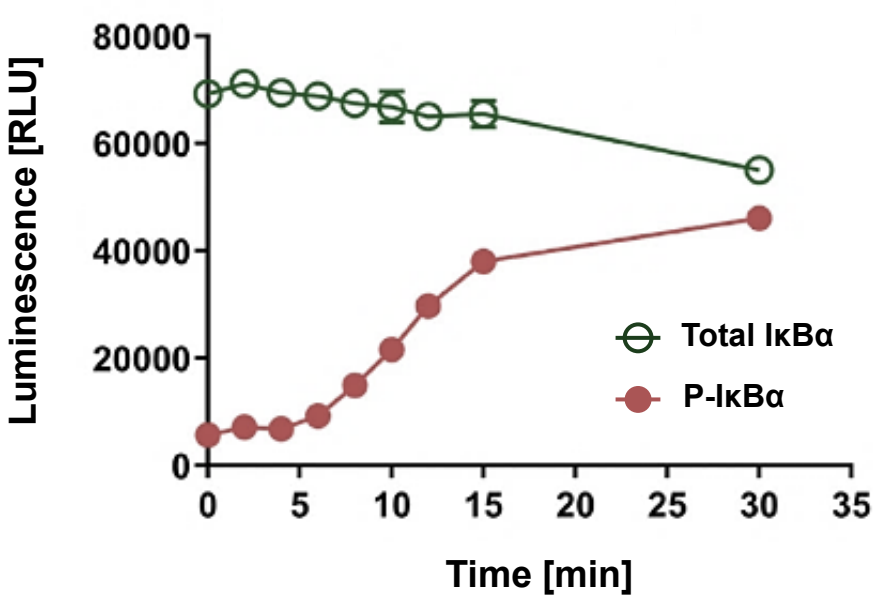
- IκBα phosphorylation at Ser32 (pS32)
- Immediately followed by rapid degradation

Cellular Signaling Pathway Analysis

NFκB Signaling Pathway



+ TNFα / MG-132



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

- Decrease in IκBα degradation
- Accumulation of phosphorylated IκBα

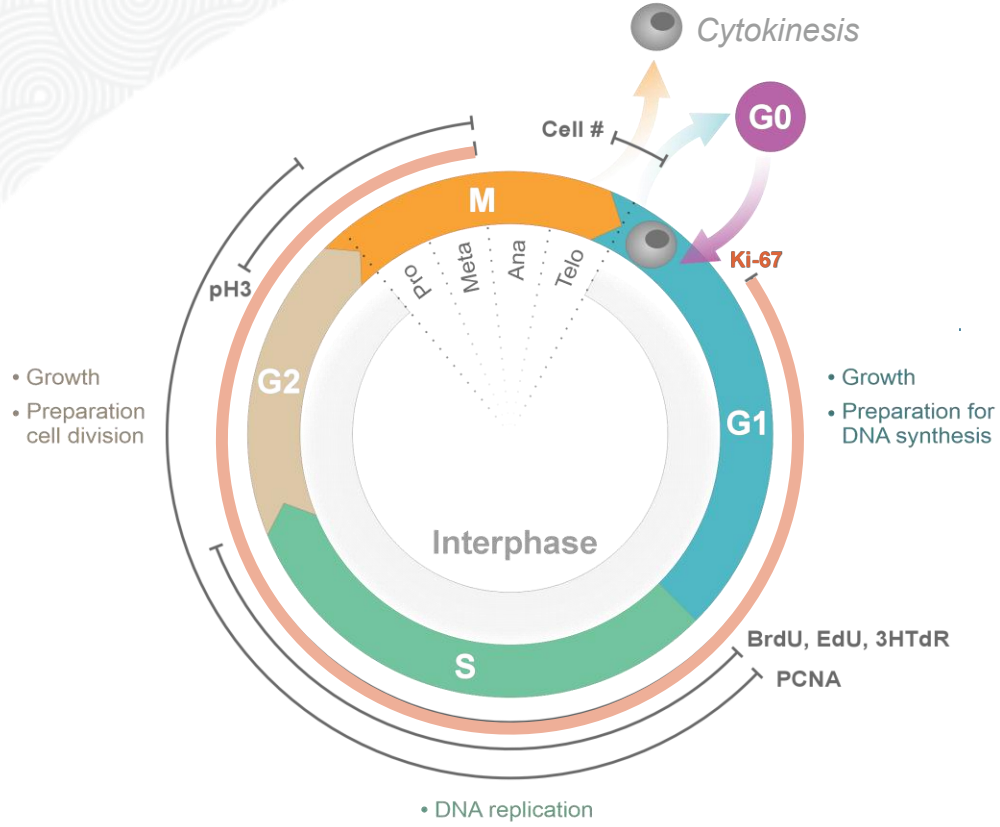
Lumit® Immunoassay Cellular Systems

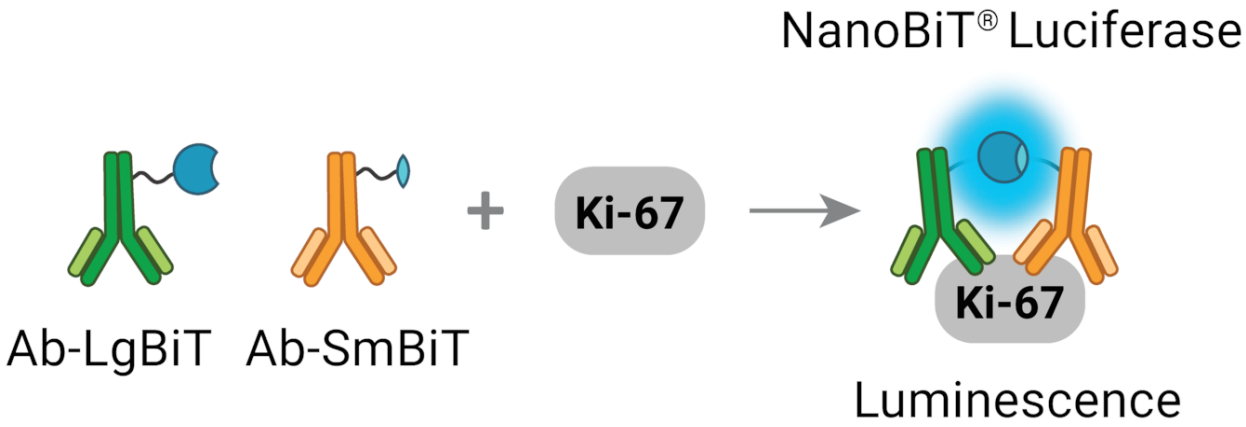
Validated with >20 phospho- and total proteins using 8 cell types, suggesting this universal immunoassay can be adapted for any pathway **with the appropriate antibodies**

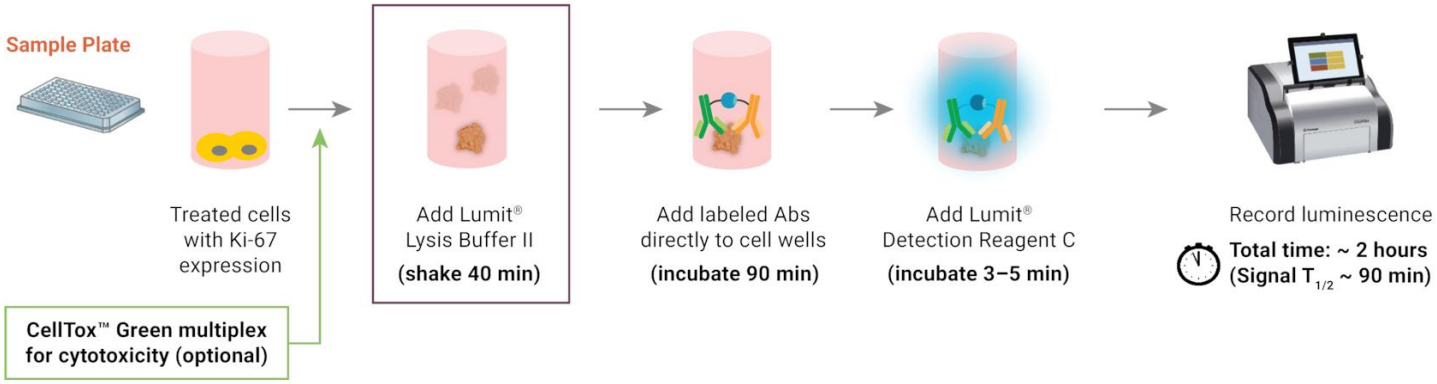
- **AKT** (**phospho-Ser473** and total protein)
- **BTK** (**phospho-Tyr223 and total protein**)
- BCL6 (total protein)
- **BRD4** (**total protein**)
- β-catenin (phospho-Thr41/Ser45 and total protein)
- **CHK1** (**phospho-Ser317**)
- c-Jun (phospho-Ser63)
- **cMET** (**phospho-Tyr1234/1235** and phospho-Tyr1349)
- CREB (phospho-Ser133 and total protein)
- **EGFR** (**phospho-Tyr1068**, phospho-Tyr1173 **and total protein**)
- Estrogen receptor (total protein)
- **ERK1** (**phospho-Thr202**)
- GSK1-3β (phospho-Ser9)
- **H2AX** (**phospho-Ser139**)
- HER2 (phospho-Tyr1196 and phospho-Tyr1221/1222)
- **IκBα** (**phosph-Ser32 and total protein**)
- **JNK** (**phosph-Thr183/Tyr185**)
- **NFκB (p65)** (**phospho-Ser536** and total protein)
- **Retinoblastoma tumor suppressor protein** (**phospho-Ser807/811** and phospho-Ser780)
- **Ribosomal protein S6** (phospho-Ser235/236, **phospho-Ser240/244**)
- Smad1 (phospho-Ser463/465 and total protein)
- Smad2 (phospho-Ser465/467 and total protein)
- **SMARCA2** (**total protein**)
- **SMARCA4** (**total protein**)
- STAT1 (phospho-Tyr701, phospho-Ser727 and total protein)
- STAT2 (phospho-Tyr690)
- **STAT3** (**phospho-Tyr705 and total protein**)

Available as Complete Assays

Lumit® hKi-67 Immunoassay for Cell Proliferation

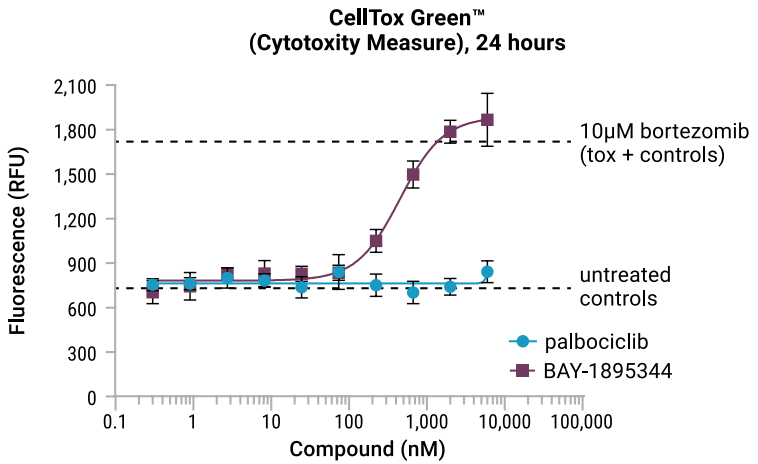


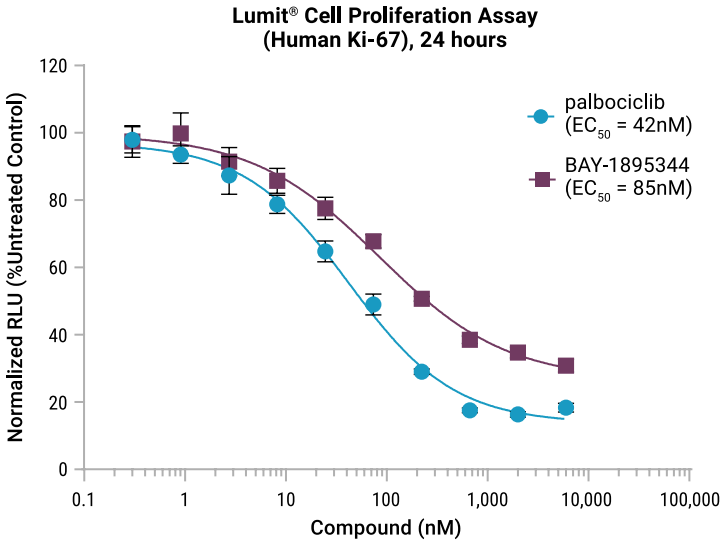


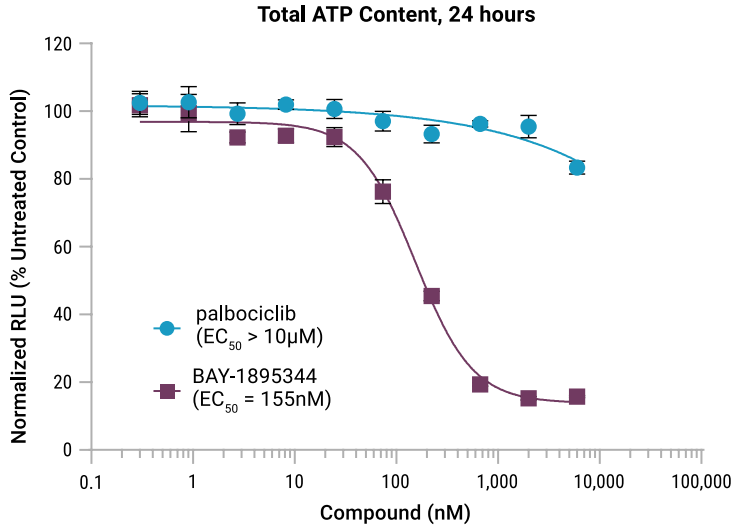


- Expressed in proliferating cells
  - Expressed in G1, S, G2 and M cell cycle phases
  - Ramps up from G1 until peaks early in M phase
- Absent in resting, non-dividing cells (G0) (quiescent, senescent, or terminally differentiated)

Lumit® hKi-67 Immunoassay for Cell Proliferation

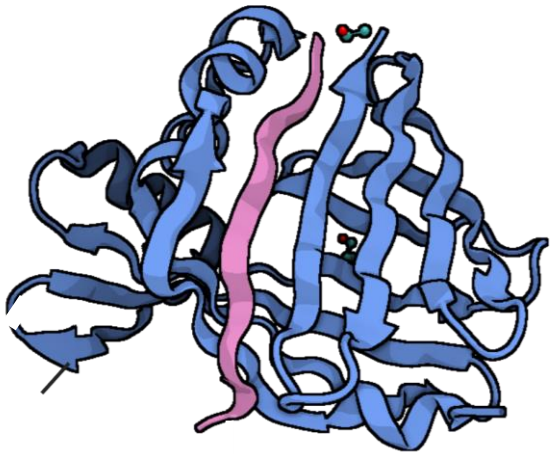




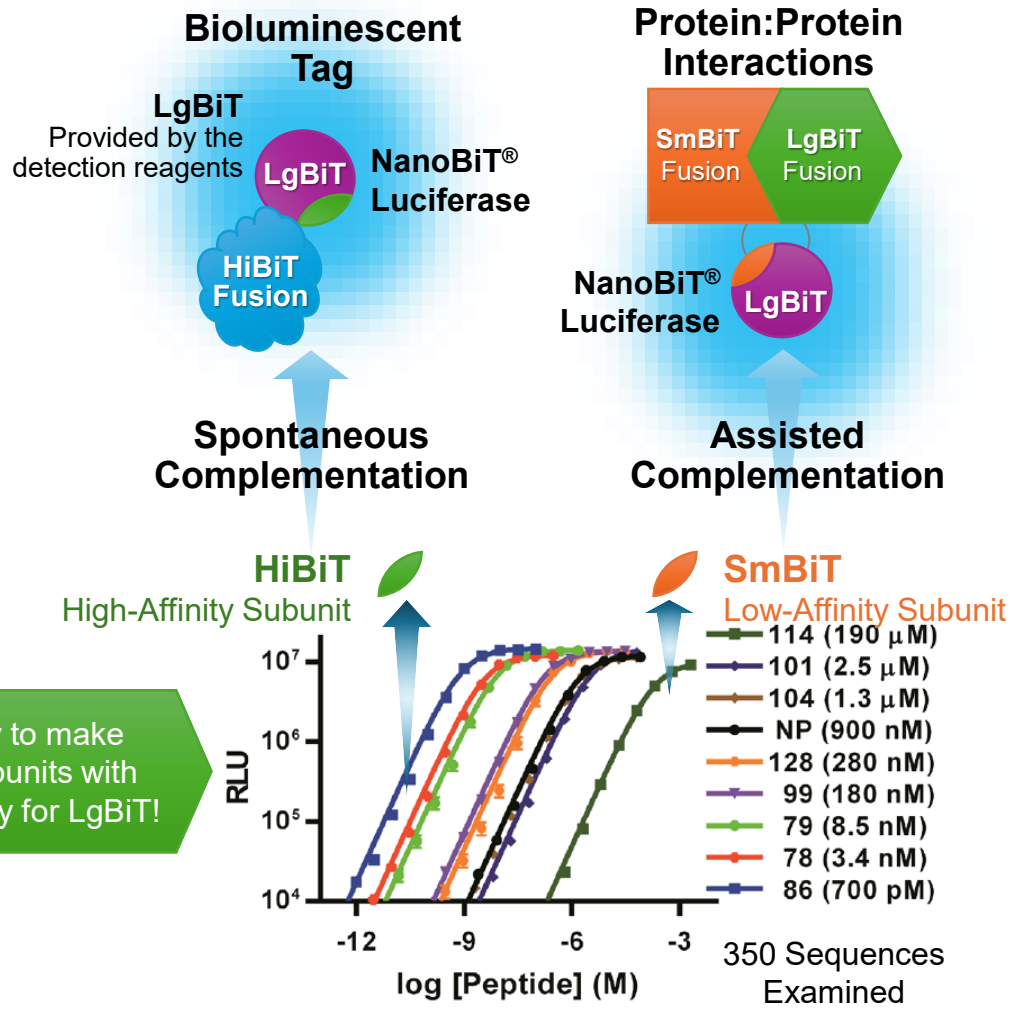


Both compounds reduced hKi-67 expression levels in a dose-dependent manner; however, BAY-1895344 induced cytotoxicity. Palbociclib produced a large change in hKi-67 levels without causing cytotoxicity after only 24 hours of treatment, enabling earlier assessment of compound effects on proliferation.

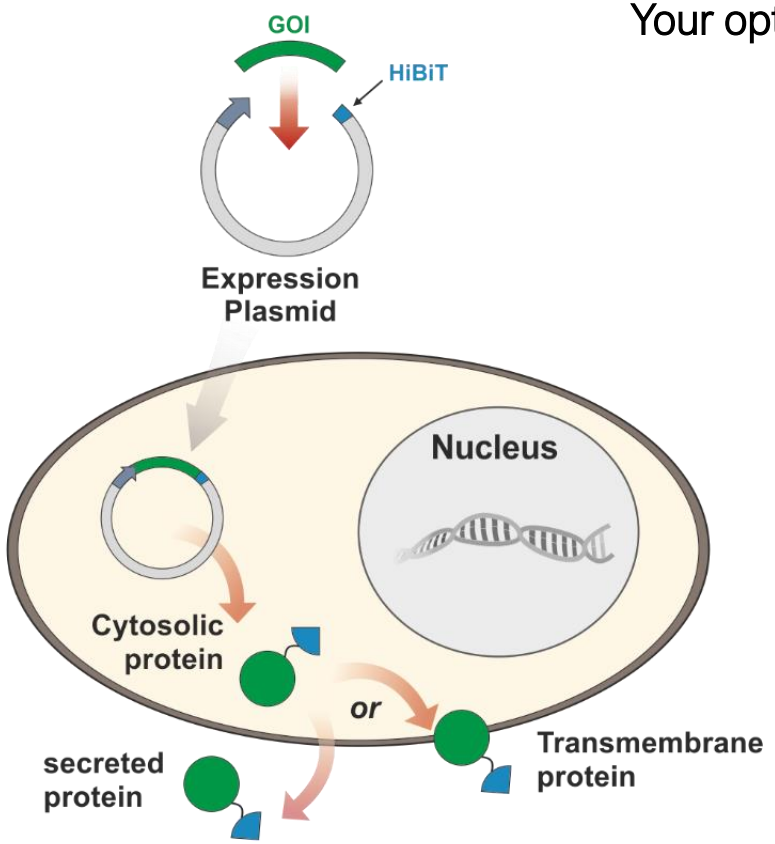
NanoLuc® Binary Technology (NanoBiT®): HiBiT



NanoLuc®



Tagging Strategy for Ectopic Expression



Your options

①

Promega's HiBiT entry vectors

- N-terminal
- C-terminal
- N-terminal + IL-6 secretion signal *
- CMV, TK, PGK

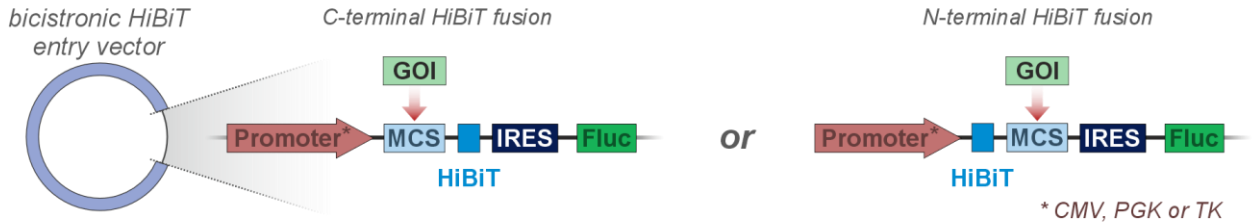
* naturally occurring secretion signals shall be removed

- Bicistronic entry vectors
  (use Fluc for normalization purposes)

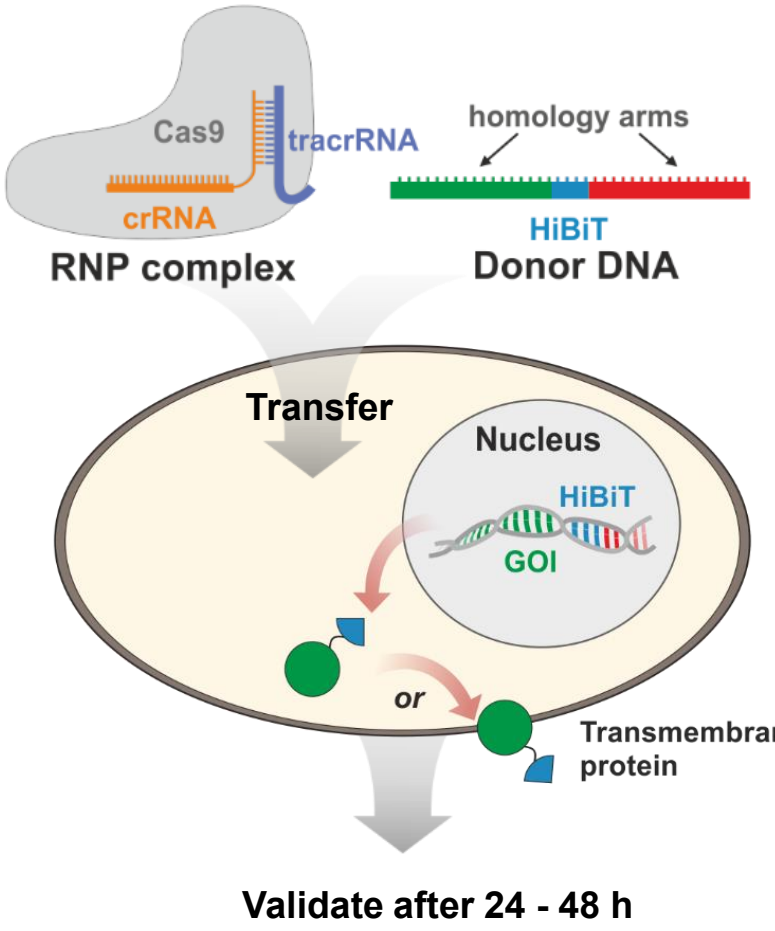
②

Use existing vector and append HiBiT via PCR amplification

(e.g. internal placement of tag)



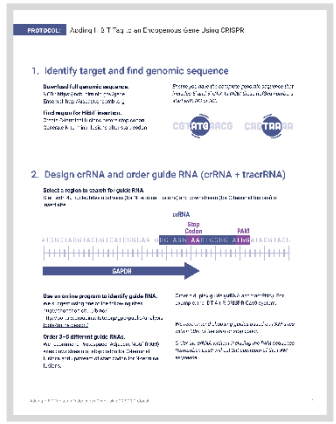
CRISPR Knock-In Strategy for Endogenous Expression



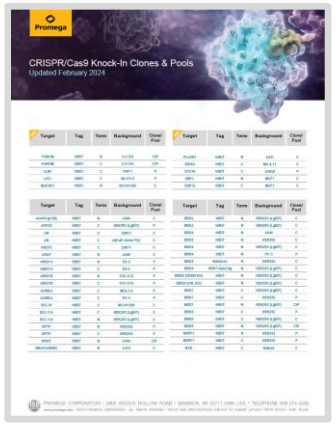
Three essential components

1. gRNA (crRNA + tracrRNA)
2. Cas9 endonuclease
3. ssDonor DNA

DIY protocol



Ready-to-use cell lines



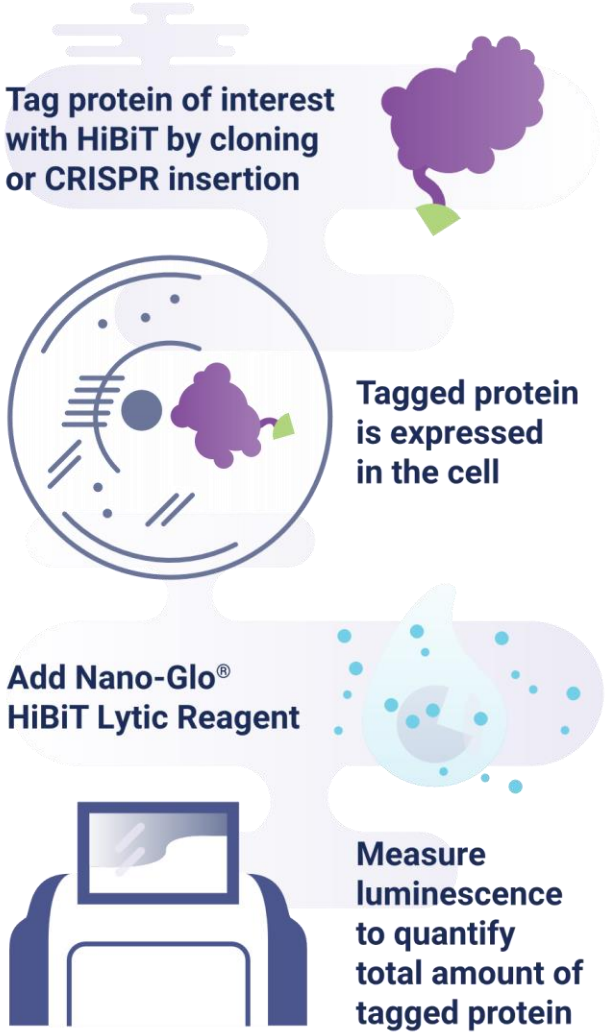
Cell line generation by licensed providers

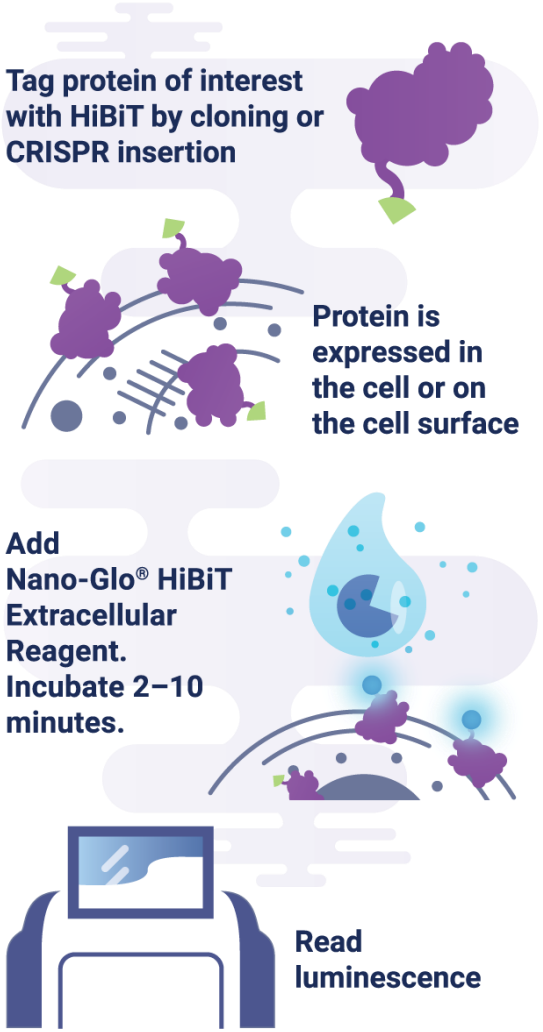
EditCo
www.editco.bio/

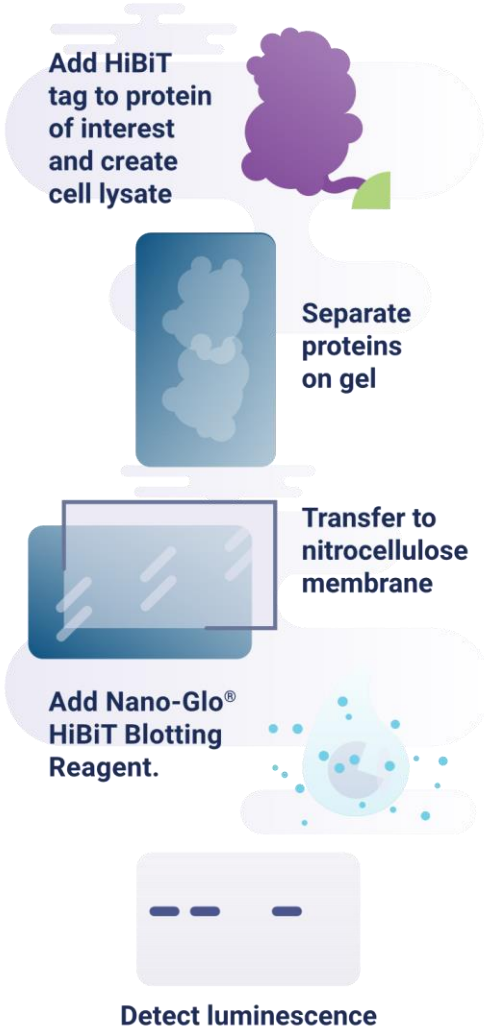
ALSTEM
CELL ADVANCEMENTS
www.alstembio.com

gRNA: guide RNA; **crRNA**: CRISPR RNA; **tracrRNA**: transactivating crRNA

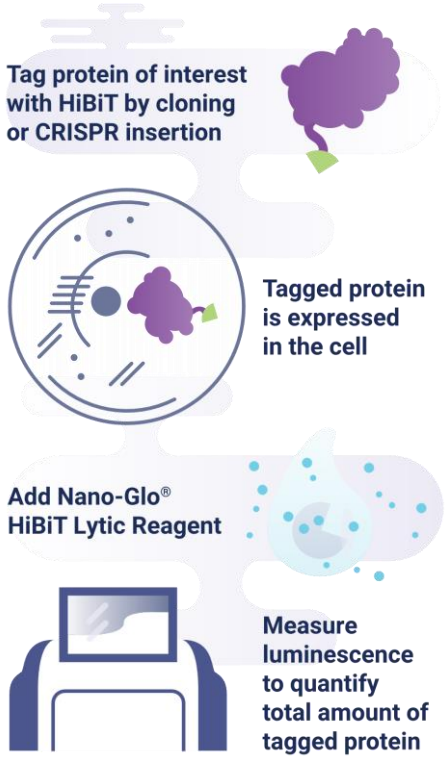
HiBiT Protein Tagging System

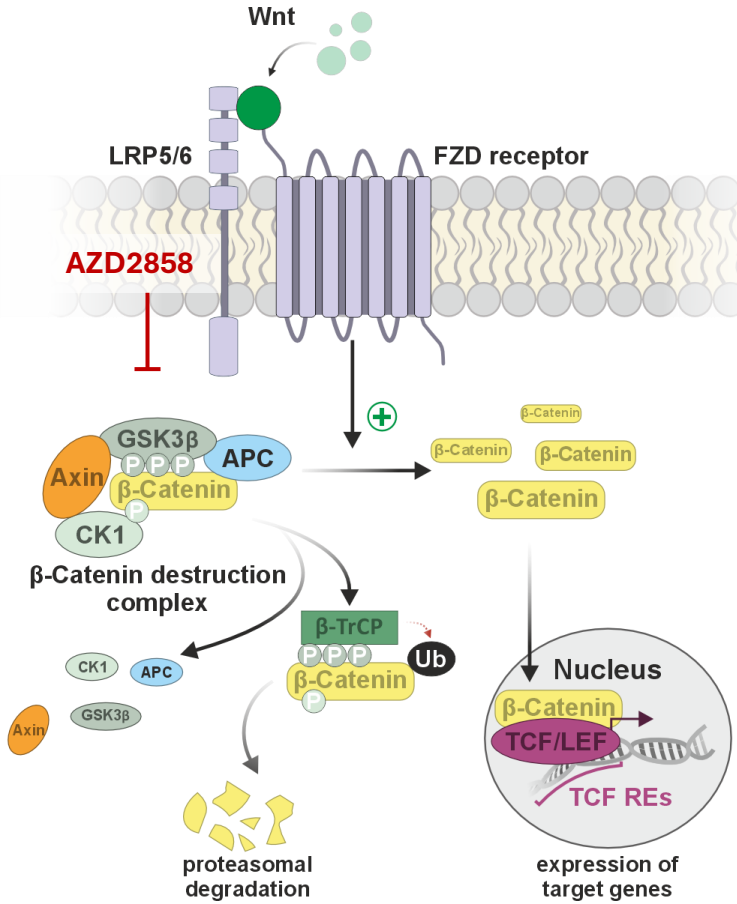


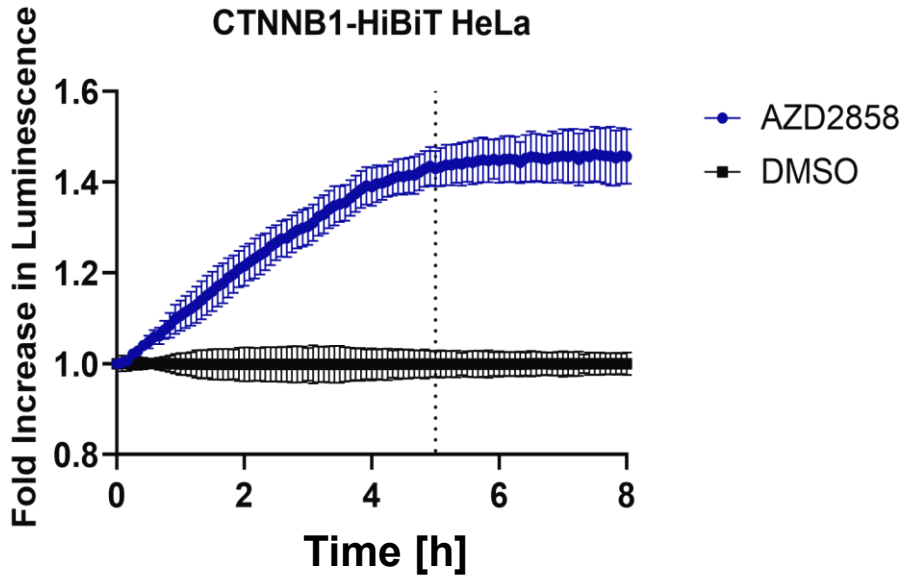




Nano-Glo® HiBiT Lytic Detection System: Measuring β-Catenin Stabilization

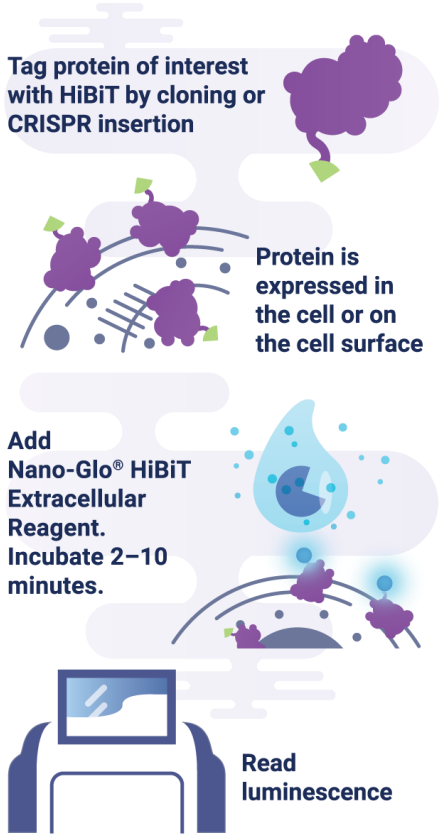


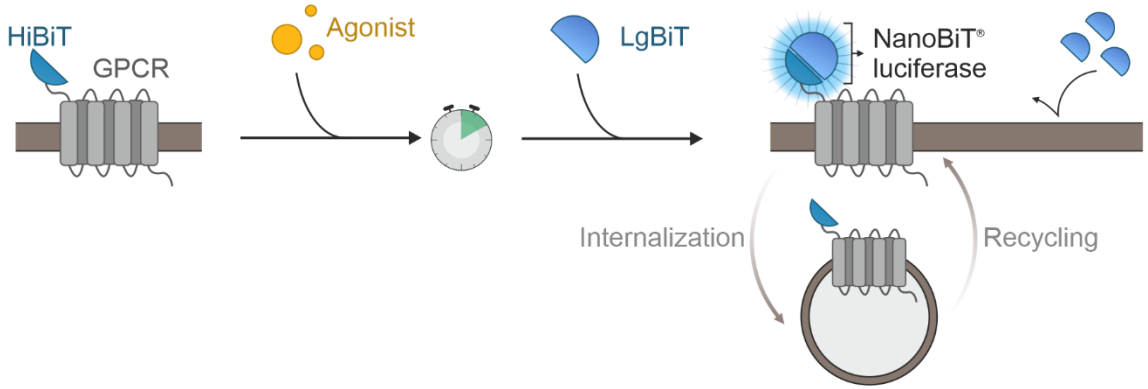


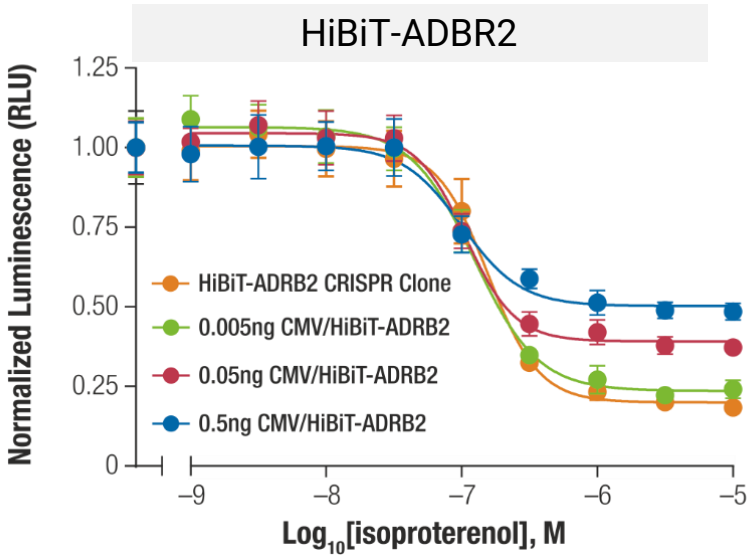


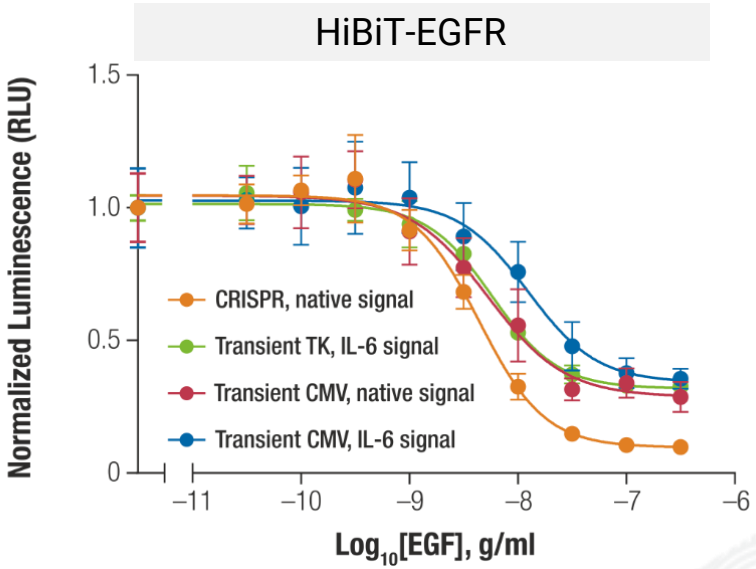
- Treatment with AZD2858 leads to modest stabilization of β-catenin
- A < 1.6-fold change at endogenous expression levels can be reliably detected

Study Receptor Internalization with HiBiT

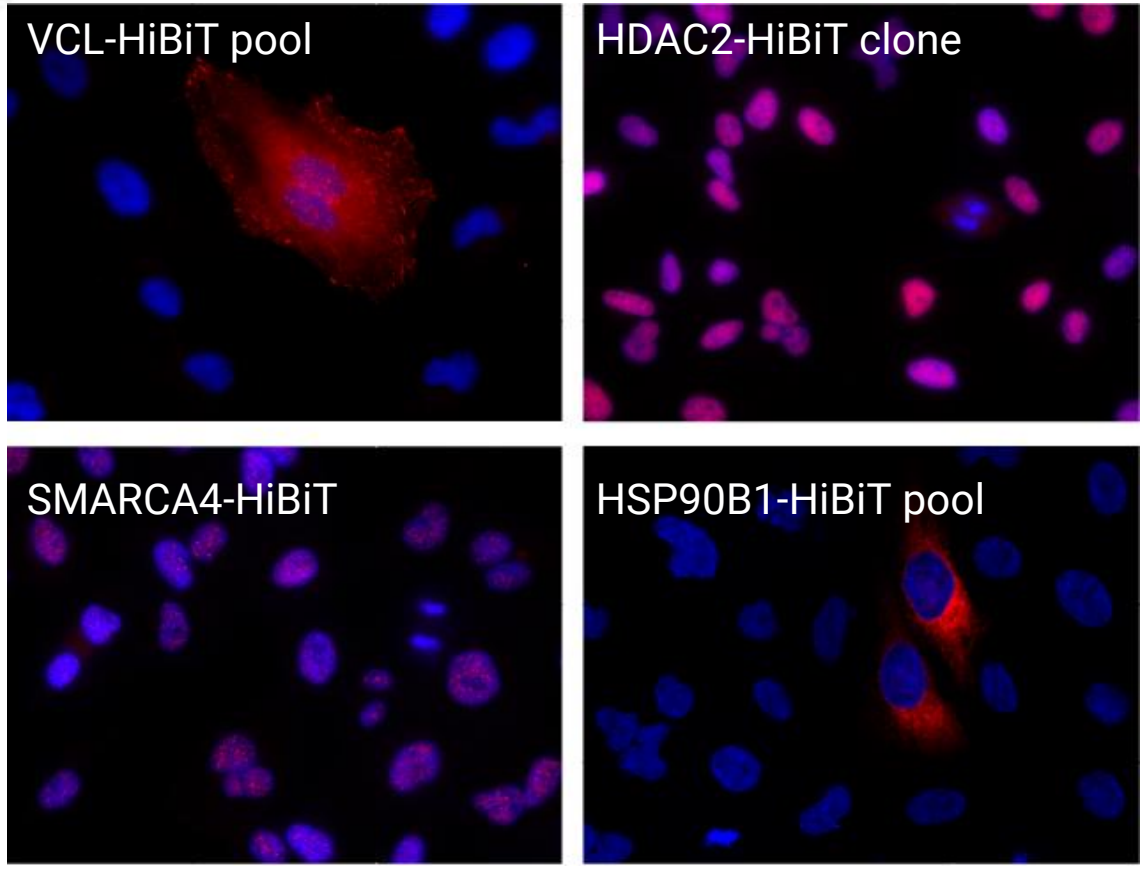




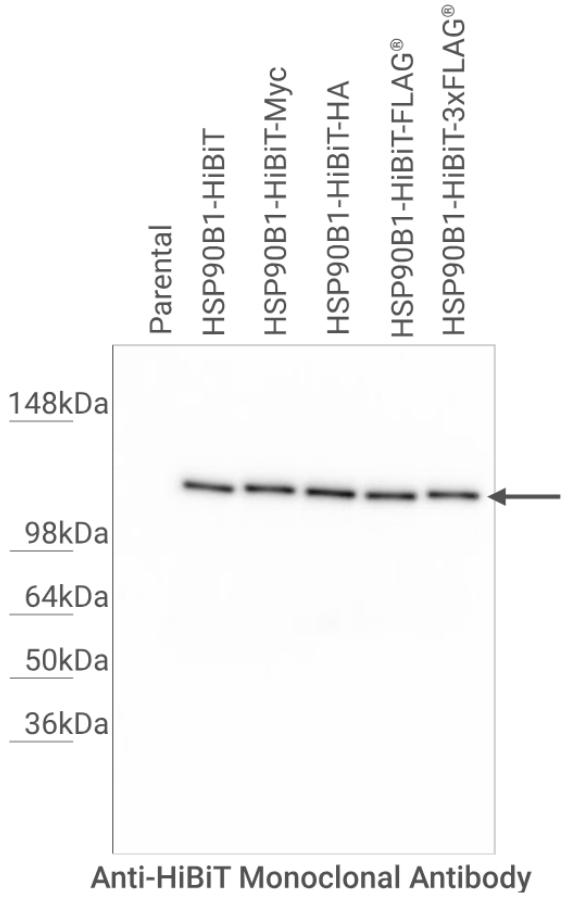




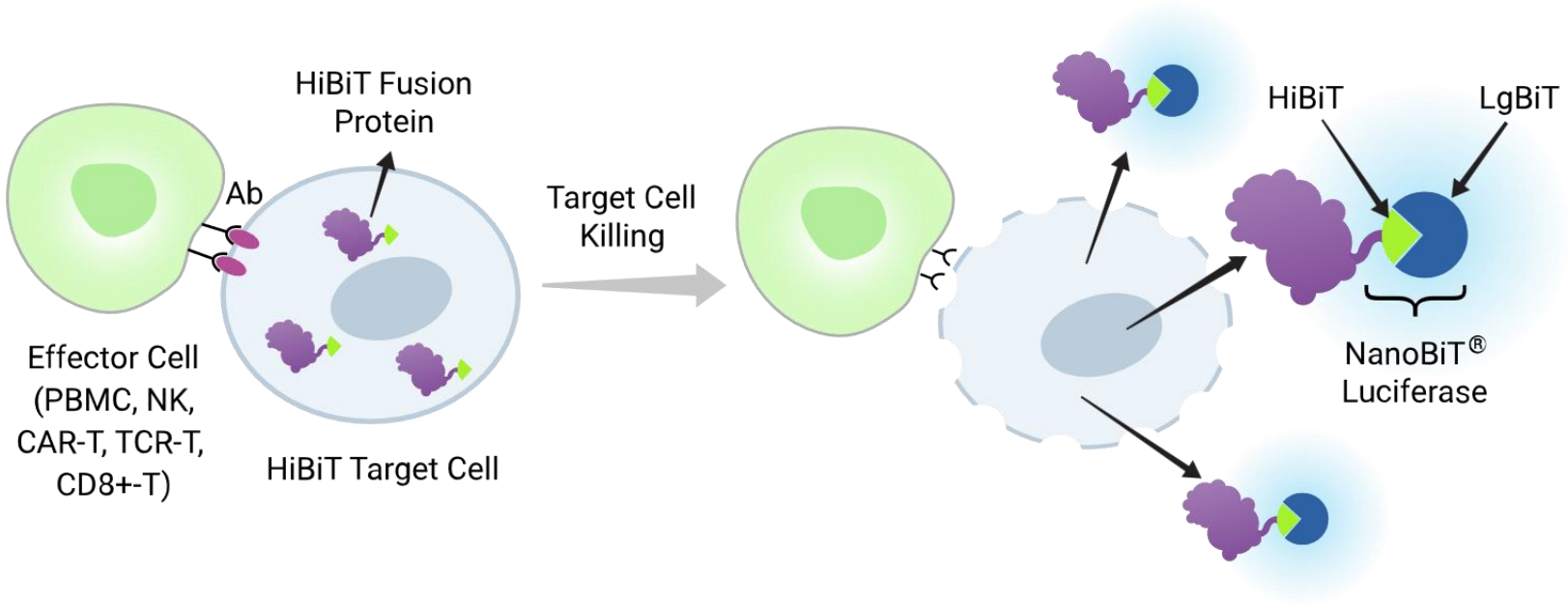
Anti-HiBiT Monoclonal Antibody



Anti-HiBiT Monoclonal Antibody (red) and Hoechst dye (blue)



Measure Cell Death of Specific Cells in Cocultures



Promega Bioluminescence Instrument Portfolio

MyGlo™ Reagent Reader

- ✓ Luminescence

GloMax® Navigator

- ✓ Luminescence
- ✓ +Injectors

GloMax® Explorer

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

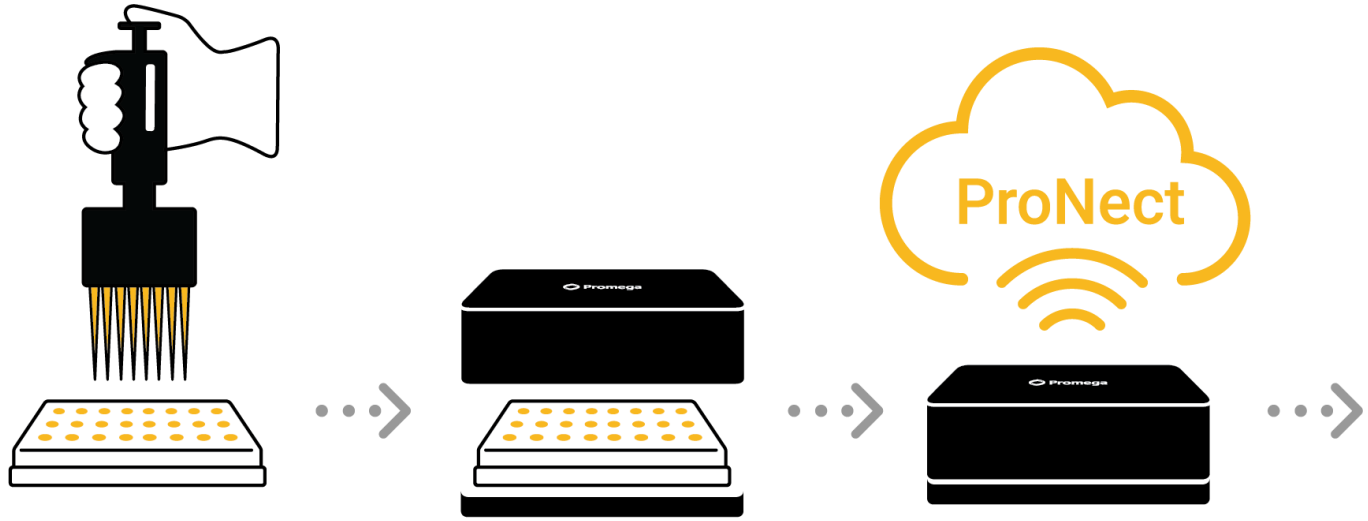
GloMax® Discover

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

GloMax® Galaxy

- ✓ NanoLuc Technology based Bioluminescence Imager

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



Questions?

For additional questions please contact:
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Let's connect!

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EASTPORT LIFESCIENCE

Promega

Thank You!
Děkuju