Bioluminescent Technologies for Studying Protein Biology and Cellular Responses

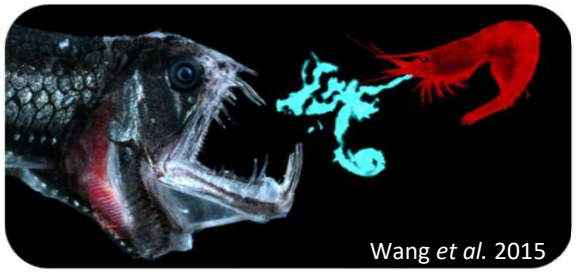
Vojtěch Ledvina, Ph.D.
vojtech.ledvina@eastport.cz

Today‘s Agenda

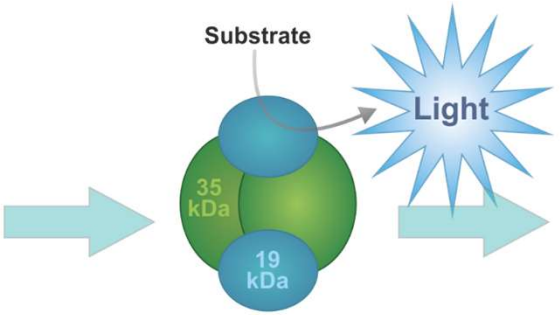
1. NanoLuc Luciferase
2. NanoBiT PPI System
3. HiBiT Protein Tagging System
4. Lumit Immunoassays
5. Multiplexing Impedance and Bioluminescence measurements

NanoLuc® Luciferase

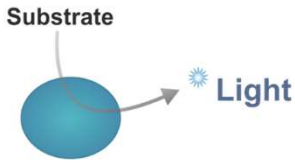
A Bright & Small Experimental Reporter



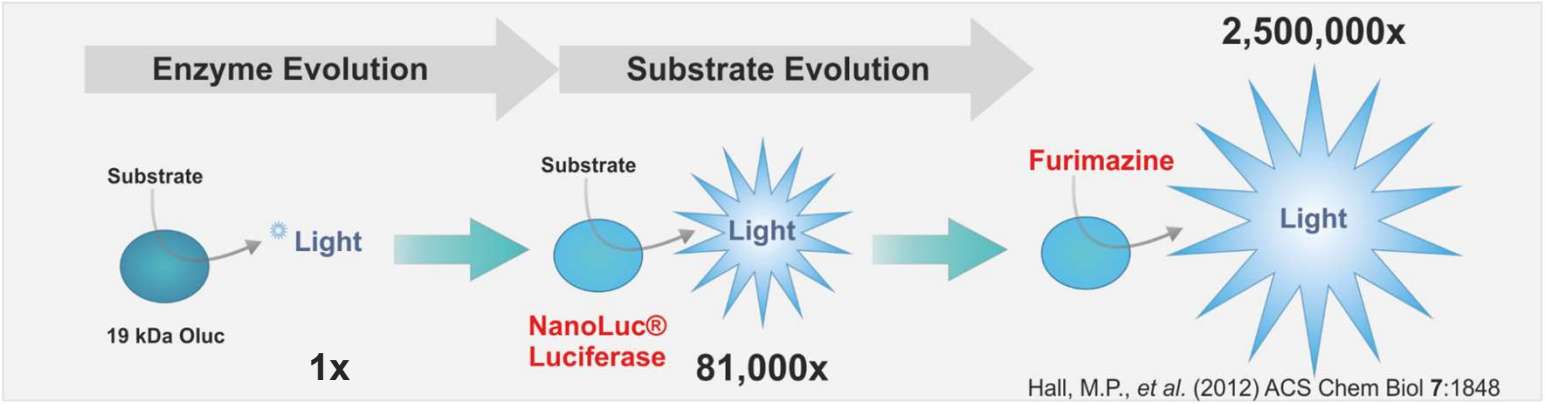
Oplophorus gracilirostris



- *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

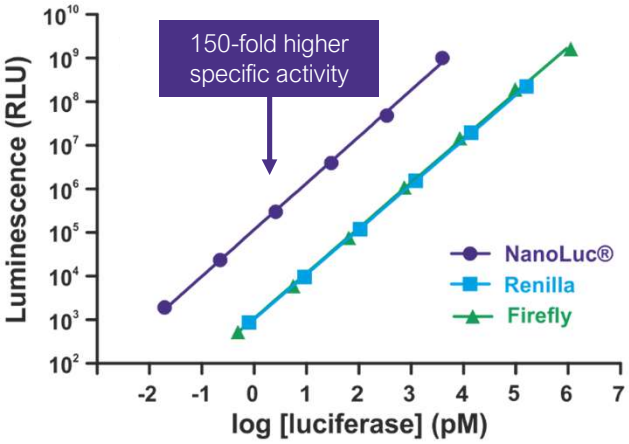


Hall, M.P., *et al.* (2012) ACS Chem Biol **7**:1848

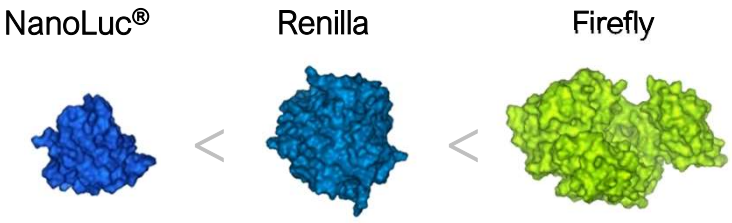
NanoLuc® Luciferase

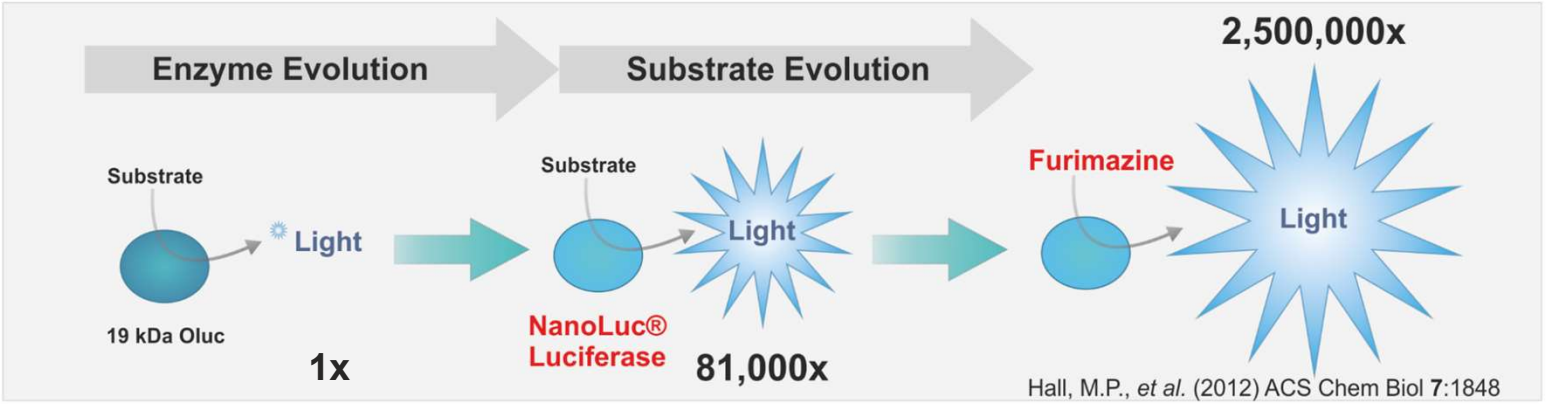
A Bright & Small Experimental Reporter

Bright, Brighter, NanoLuc®



Small, Smaller, NanoLuc®

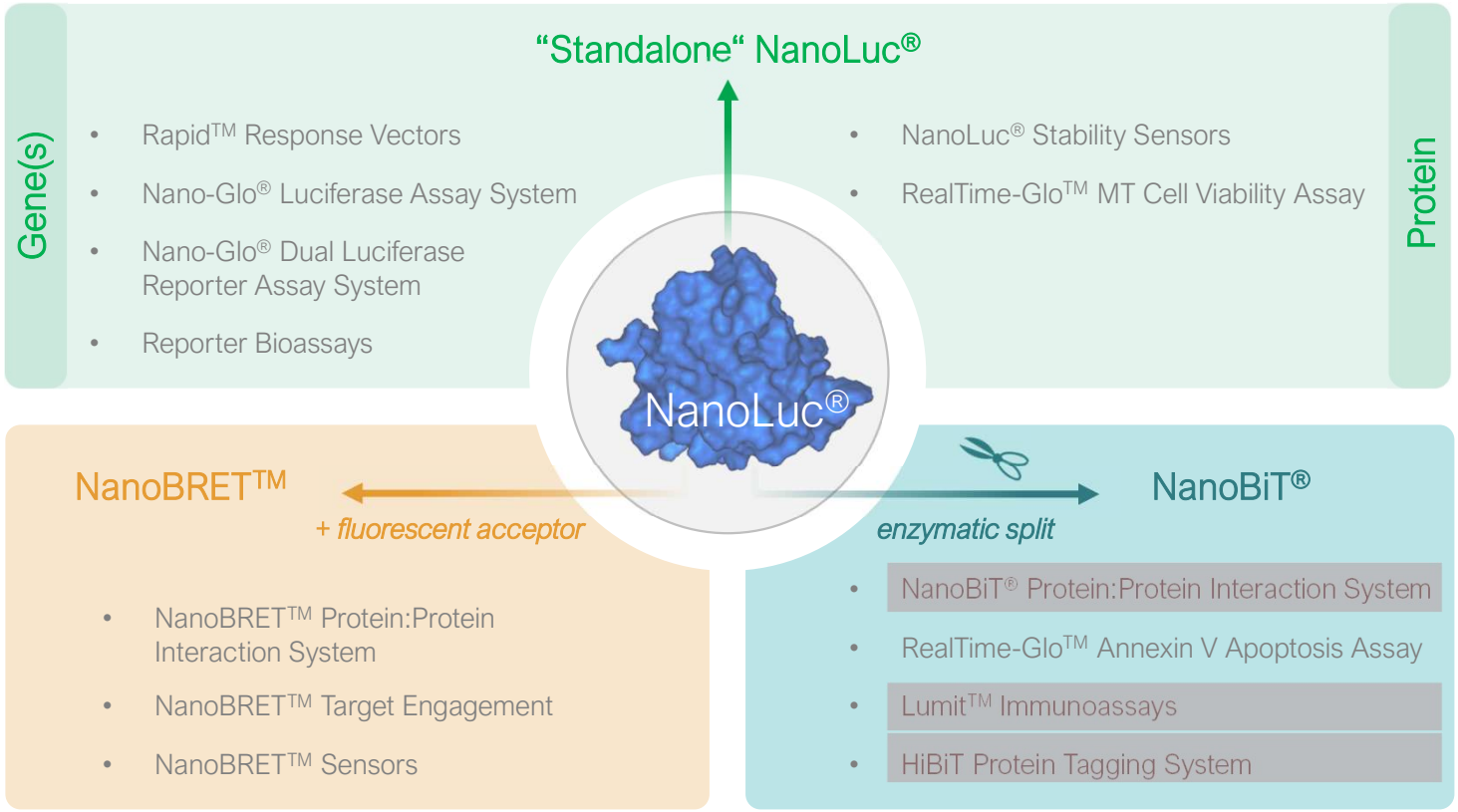




Hall, M.P., *et al.* (2012) ACS Chem Biol **7**:1848

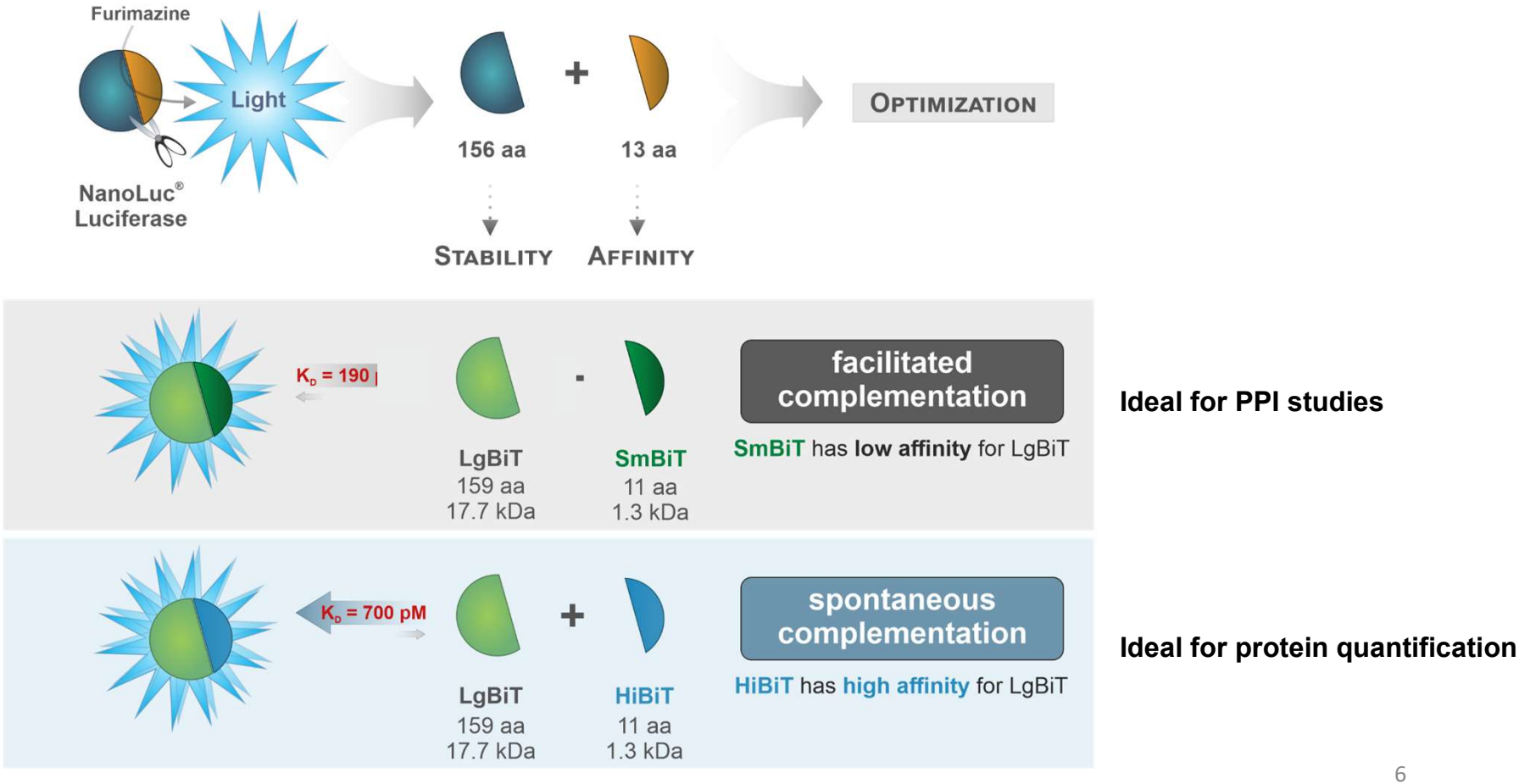
NanoLuc® Luciferase Technologies

Your Companion to Study Cellular Biology



NanoLuc® Binary Technology (NanoBiT®)

A Structural Complementation Reporter Designed for Biomolecular Interaction Studies

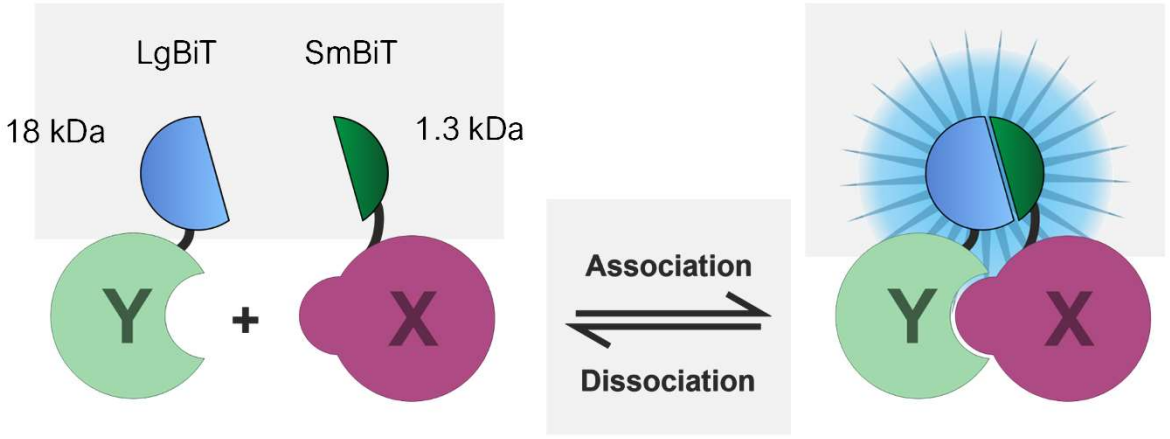


NanoBiT Protein:Protein Interaction System

Investigate Interaction Dynamics in Live Cells

Small tag size
minimal influence on fusion partner

Bright signal upon complementation
enables low expression levels

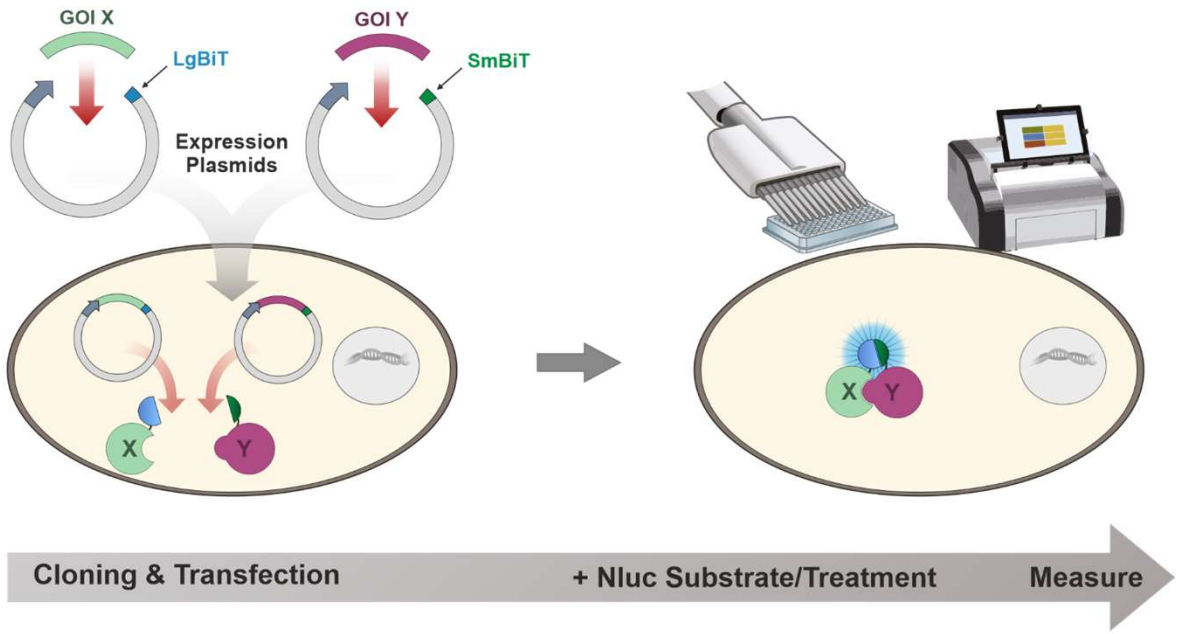


Low intrinsic affinity
reversible to allow investigation of PPI dynamics

increased signal specificity

NanoBiT PPI Workflow

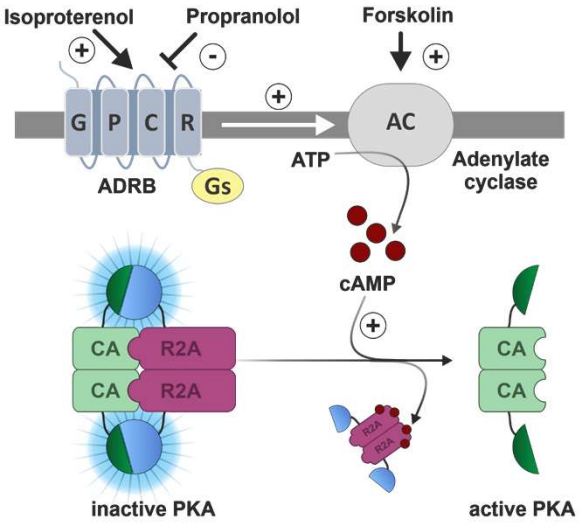
A Simple Transfection-based Experiment

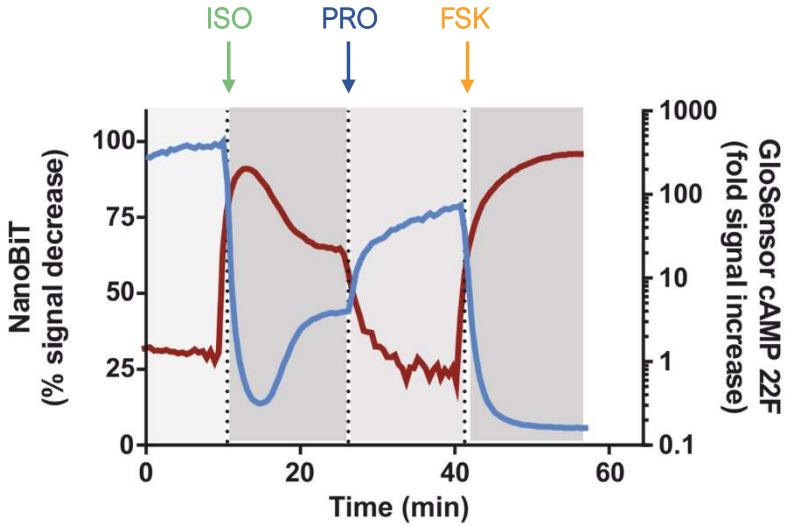


1. Determine optimal LgBiT/SmBiT combinations that shows maximal fold signal change
   tool compound versus vehicle control or in comparison to HaloTag®-SmBiT negative control
2. Check for signal specificity
   expected response to tool compound or signal of SmBiT/LgBiT fusions 10 – 1,000-fold higher than LgBiT fusion co-expressed with HaloTag®-SmBiT (general guideline)

Validation of NanoBiT PPI

The Protein Kinase A Model

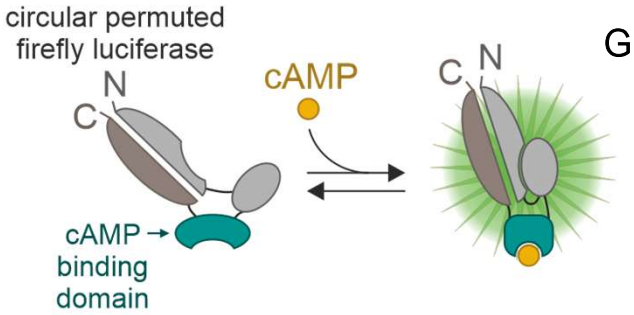




Isoproterenol (ISO)
ADRB agonist (cAMP ↑)

Propranolol (PRO)
ADRB antagonist (cAMP ↓)

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



GloSensor™

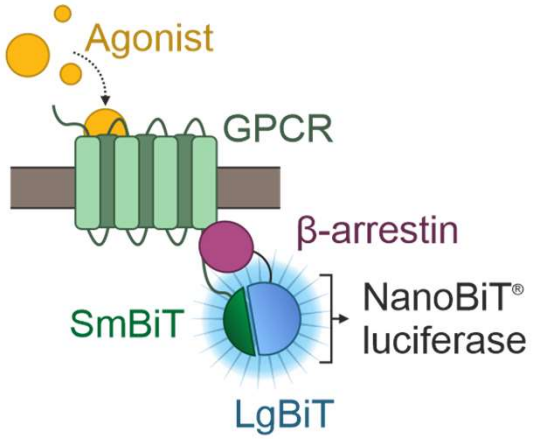
Conclusions

- Endogenous biology is maintained with the NanoBiT® PPI System
- The NanoBiT® PPI System functions in a reversible manner

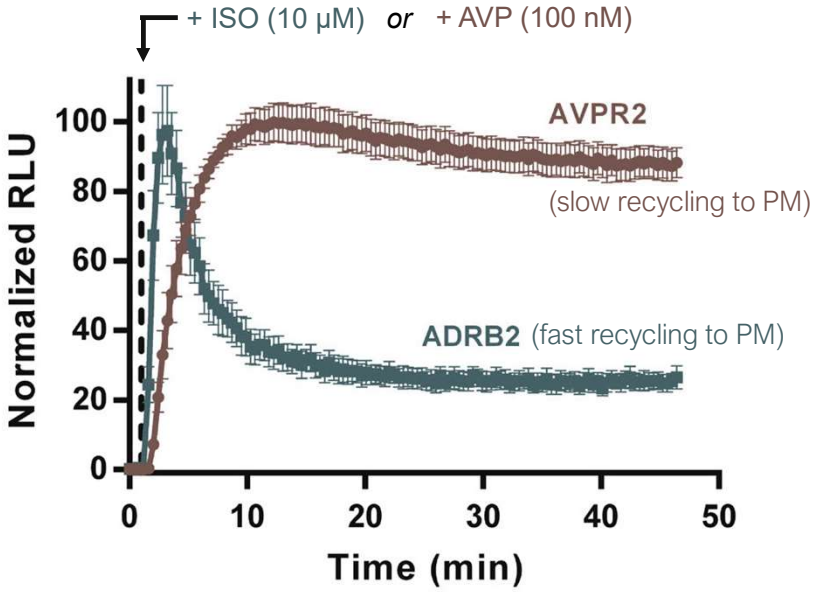
Dixon et al. *ACS Chem. Biol.* 2016, 11, 2, 400–408.

Validation of NanoBiT® PPI

β-Arrestin Recruitment to GPCRs



ADRB2-LgBiT:SmBiT-ARRB2
AVPR2-SmBiT:LgBiT-ARRB2

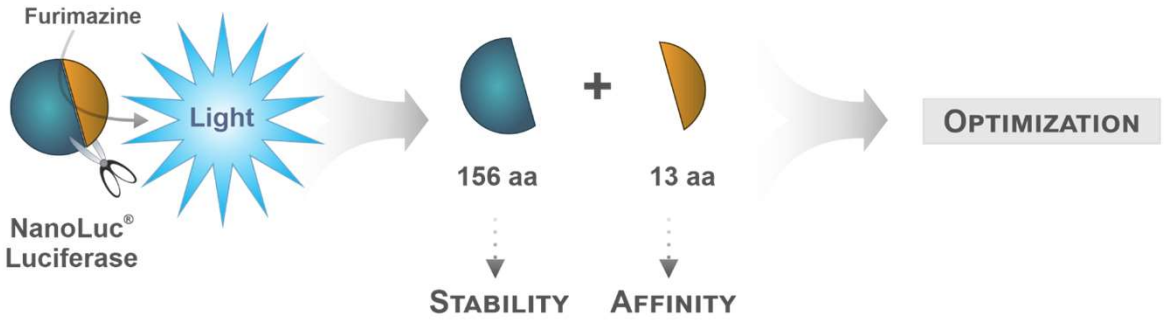


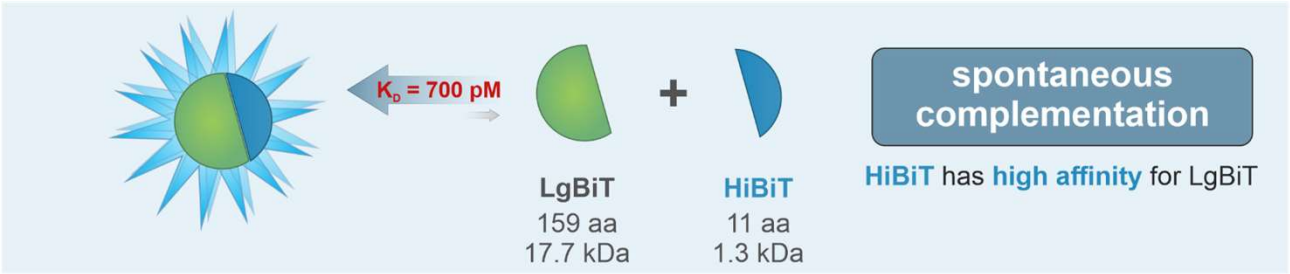
Modified from Dixon, AS. *et al.* (2015) ACS Chem Biol. 11, 2, 400–408

- ADRB2:ARRB2 signal is more transient than AVPR2:ARRB2 signal
- NanoBiT can be used to monitor transient PPIs in real-time

NanoLuc® Binary Technology (NanoBiT®)

A Structural Complementation Reporter Designed for Biomolecular Interaction Studies

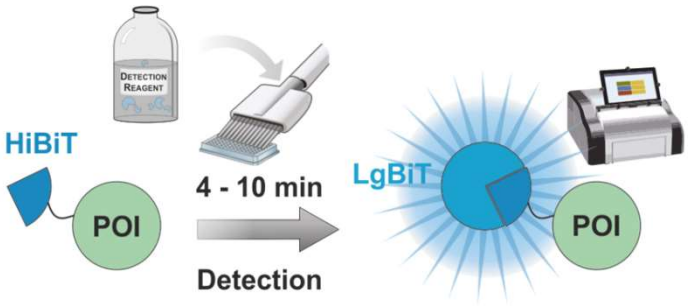


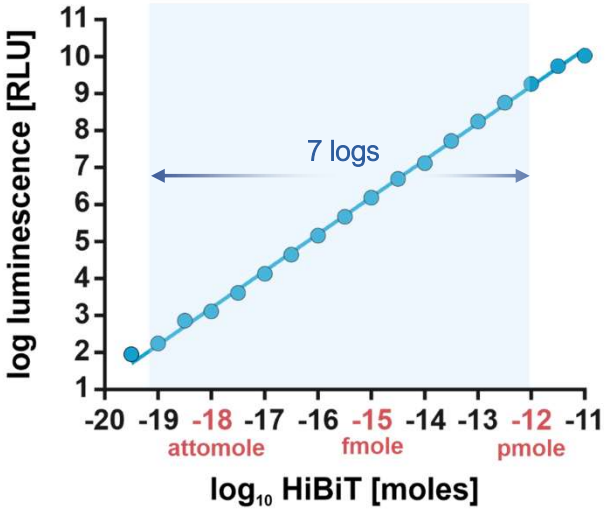


Ideal for protein quantification

HiBiT Protein Fusion Tagging System

Principle & Features





Small Tag Size (11 aa, 1.3 kDa)

- Low risk artificially affect fusion partner

Easy Knock-in with CRISPR

- Work at native expression level
- Maintain transcriptional regulation
- Avoid gene dosage effects

Simple, Flexible & Rapid Detection

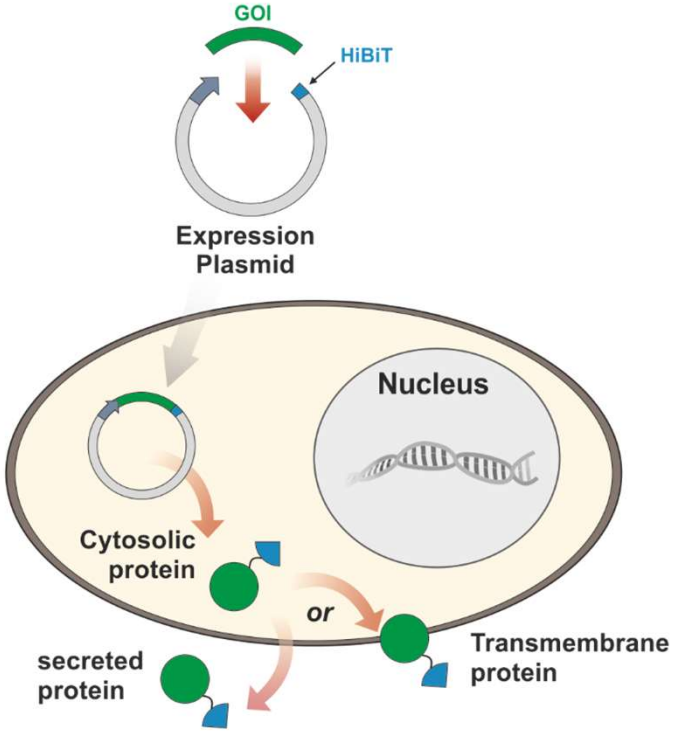
- Homogenous 1-step assay („add only“)
- No antibodies and no washing steps required
- Amenable to HTS and easy to automate

Sensitive & Quantitative

- Sub-attomolar levels can be detected
- High linear range of >7 logs

Strategies for Tagging with HiBiT

Ectopic Expression Using Constitutive Promoter-driven Plasmid



Your options

1 Promega's HiBiT entry vectors

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

* naturally occurring secretion signals shall be removed

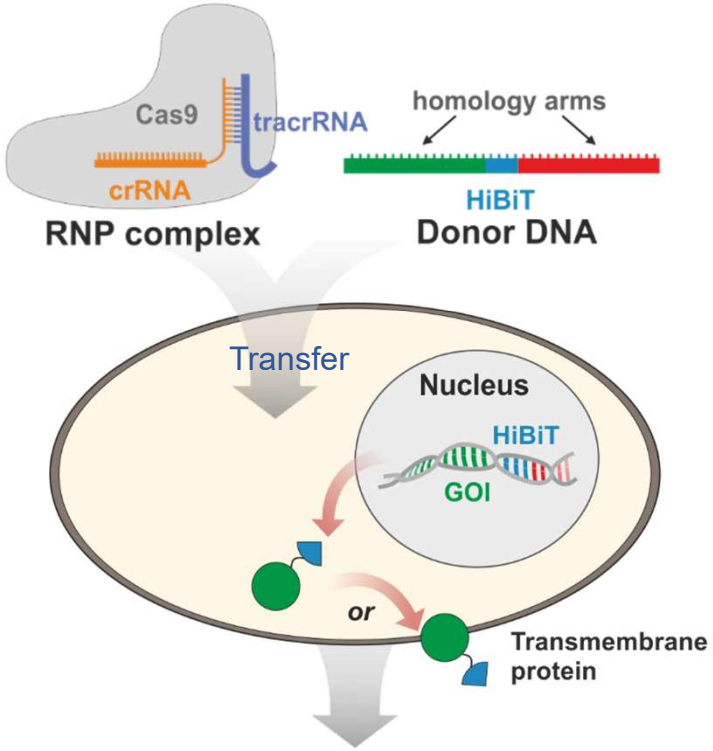
- Bicistronic entry vectors
  (use Fluc for normalization purposes)

2 Use existing vector and
append HiBiT via PCR amplification

(e.g. internal placement of tag)

Strategies for Tagging with HiBiT

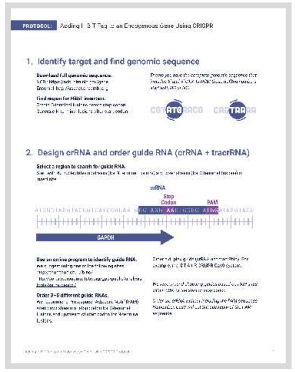
Endogenous Expression Following CRISPR-mediated Tagging



Three key components

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

DIY protocol



Ready-to-use cell lines

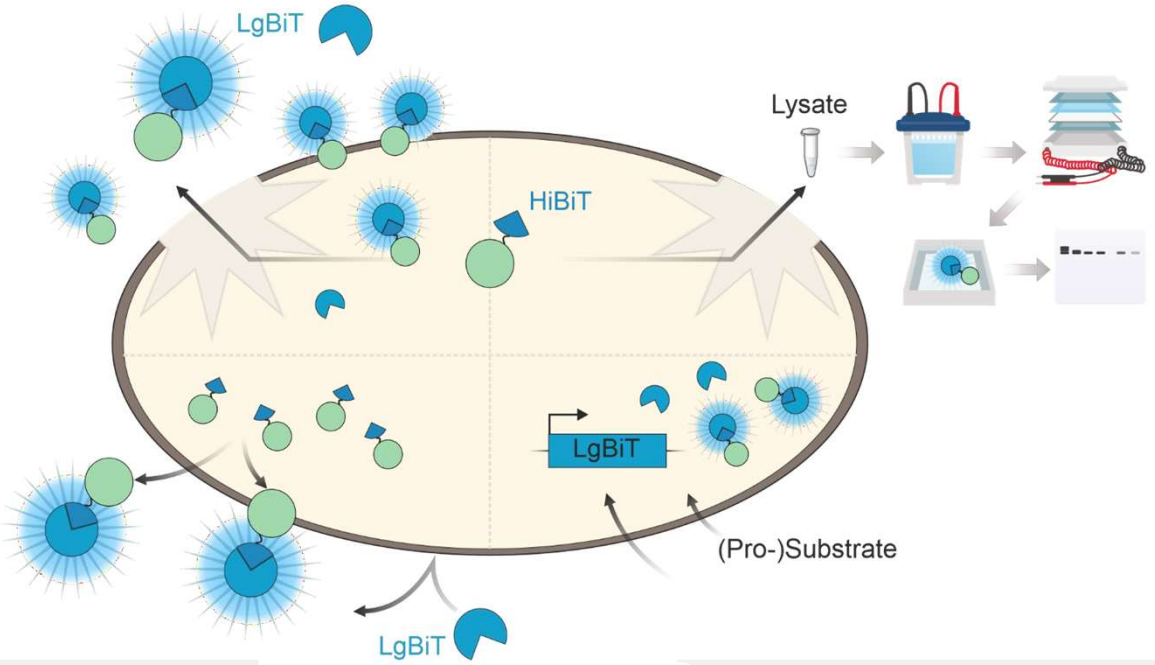
gRNA: guide RNA; **crRNA**: CRISPR RNA; **tracrRNA**: transactivating crRNA

Detection of HiBiT Fusion Proteins

Choose From Different HiBiT Detection Strategies

1 Nano-Glo® HiBiT Lytic Detection System

2 Nano-Glo® HiBiT Blotting System



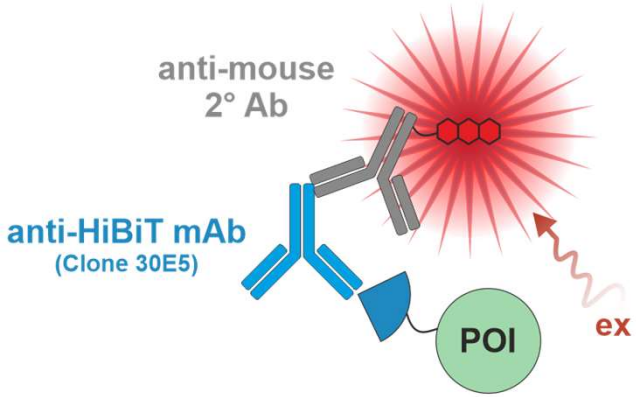
3 Nano-Glo® HiBiT Extracellular Detection System

4 LgBiT co-expression Nano-Glo® Live Cell Substrate

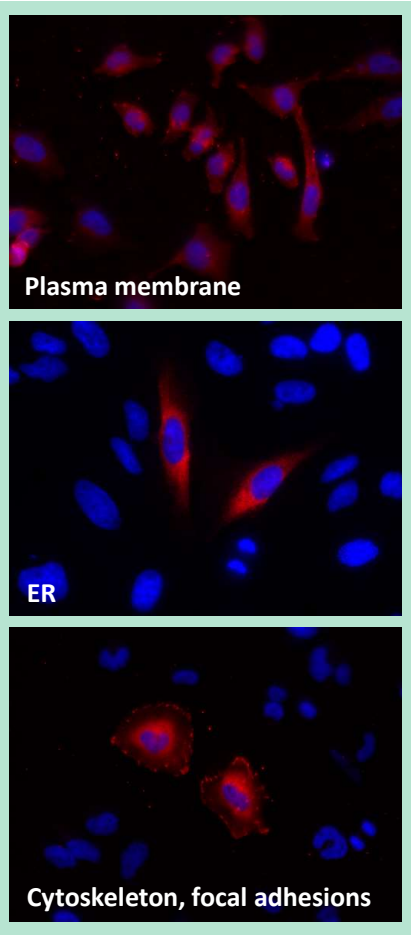
Time course analysis up to 72 hours

Immunodetection of HiBiT Proteins

Immunofluorescent Imaging



Hoechst dye
AlexaFluor® 647



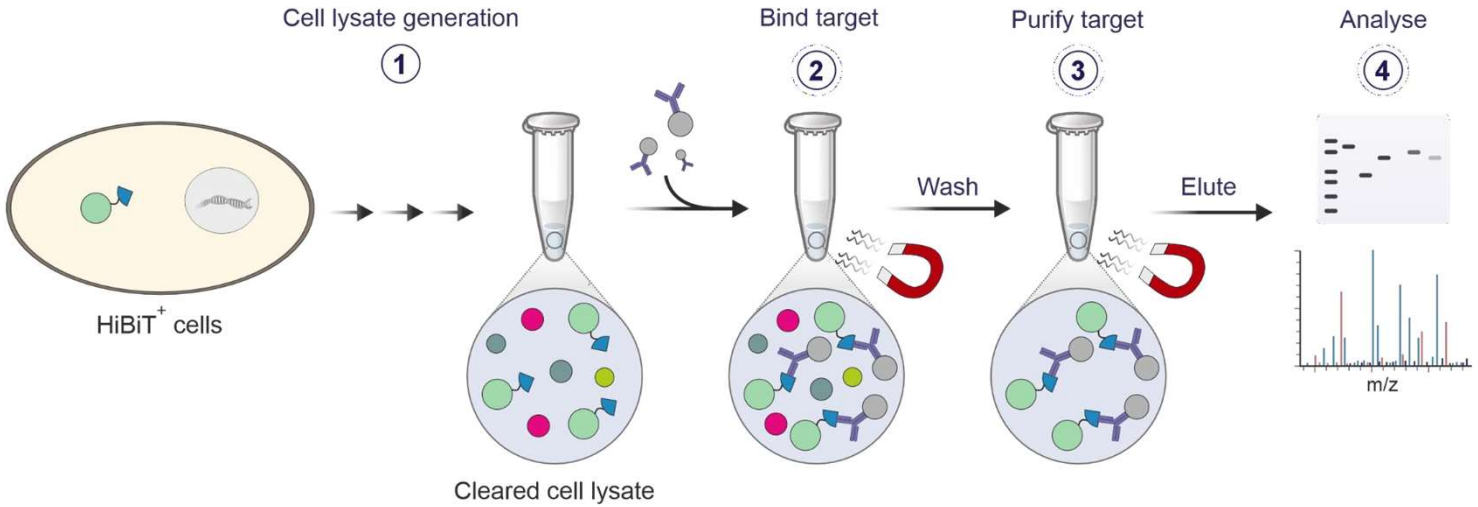
FACTS

- Potent mAb directed against HiBiT tag
- Validated for various applications including:

✓ Immunofluorescence (NEW anti-HiBiT pre-conjugated to Green488 or FarRed647)
✓ Western blotting
✓ Immunoprecipitation (NEW anti-HiBiT Magne® Beads)
✓ FACS

Anti-HiBiT Magne® Beads

Workflow



- A cleared cell lysate is generated from HiBiT+ cells
- Lysate is incubated with Anti-HiBiT Magne® Beads over night at 4°C or >30 min at RT
- Elution can be performed with

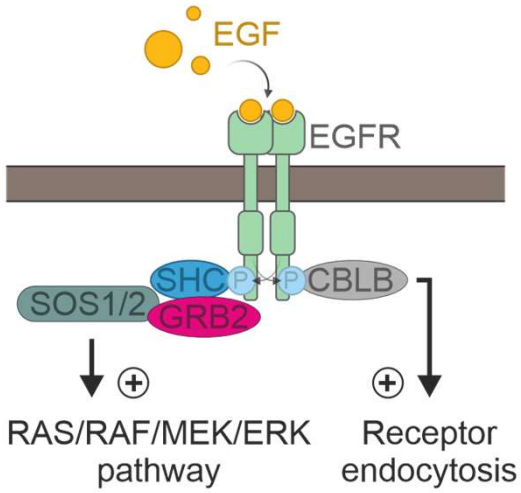
(1) SDS loading buffer and heating to 70 °C for 10 min

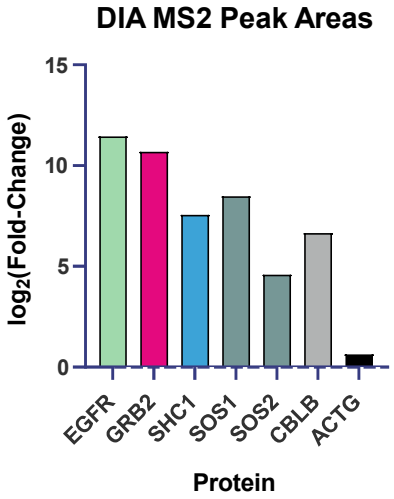
(2) Glycine-HCl (pH 2.5) at RT for 5 – 10 min

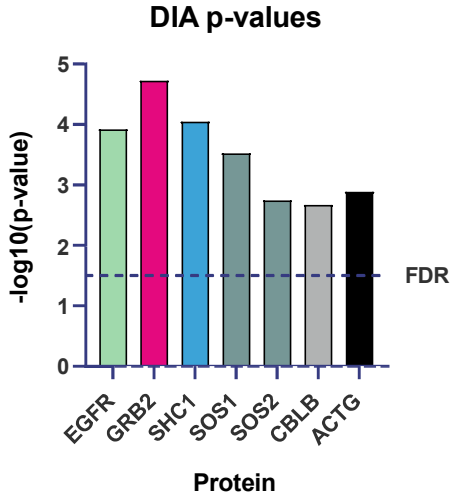
(3) DrkBiT peptide overnight at 4°C

Anti-HiBiT Magne® Beads

Workflow



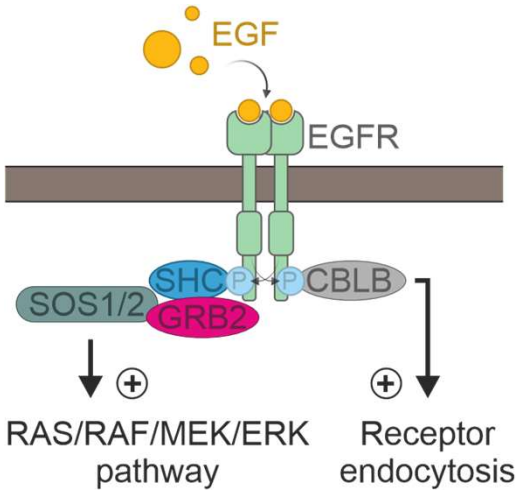


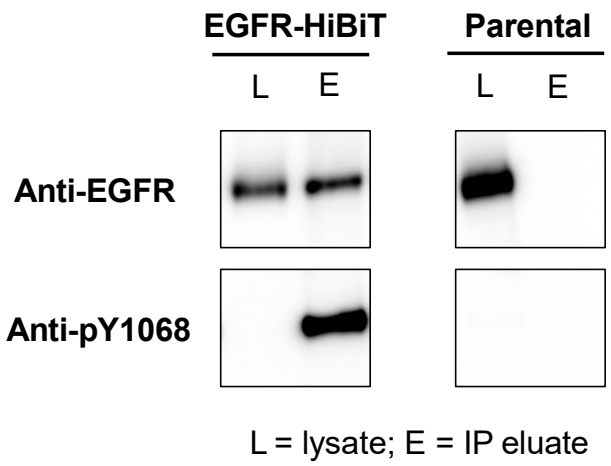


- EGFR-HiBiT HeLa CRISPR knock-in cells
- Upon EGF stimulation, co-IP was performed using the Anti-HiBiT Magne® Beads
  - ✓ DIA MS of IP eluates showed enrichment of EGFR and known direct/indirect interactors
  - ✓ EGFR enrichment and phosphorylation was confirmed by Western blot analysis
  - ✓ FACS

Anti-HiBiT Magne® Beads

Workflow

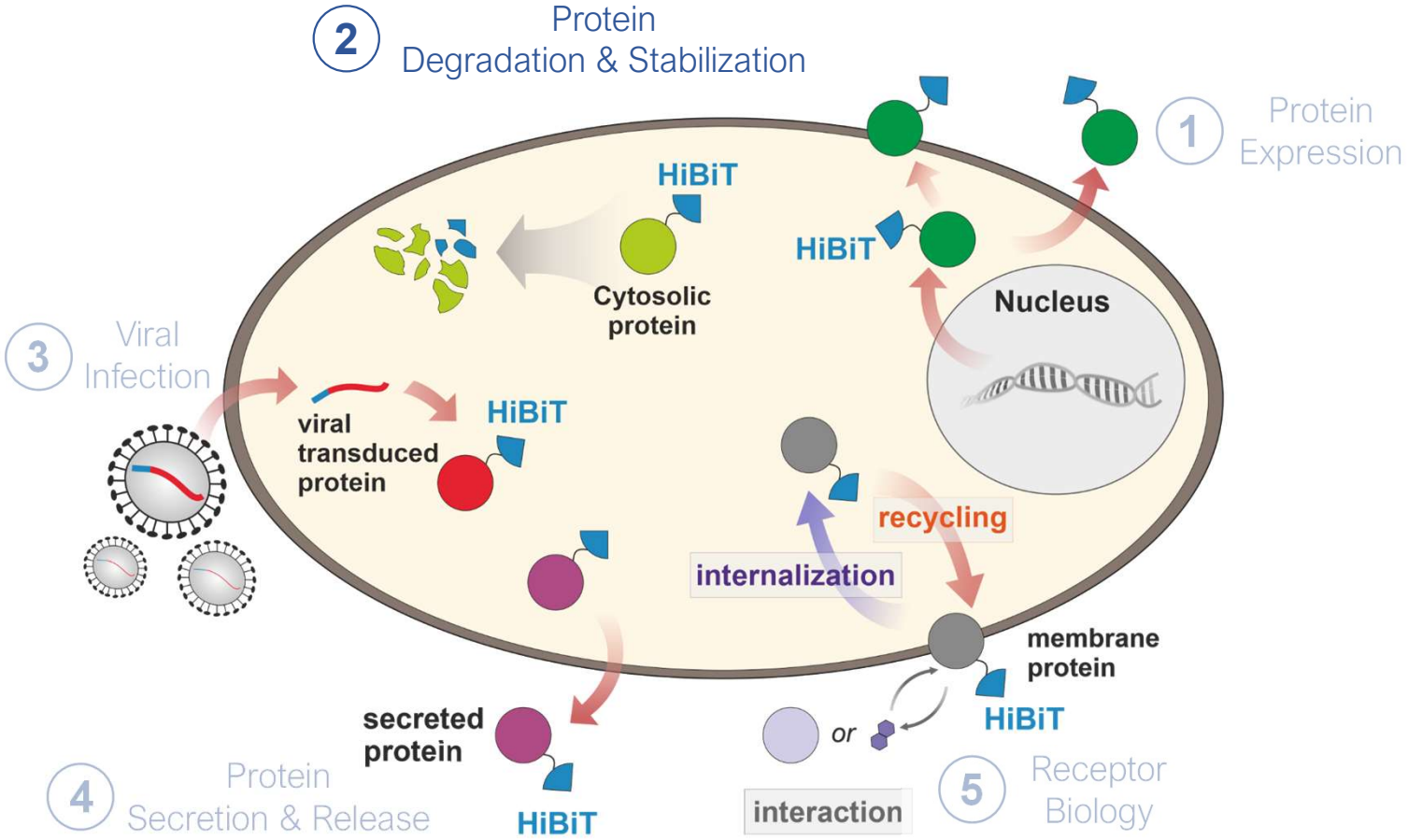




- EGFR-HiBiT HeLa CRISPR knock-in cells
- Upon EGF stimulation, co-IP was performed using the Anti-HiBiT Magne® Beads
  - ✓ DIA MS of IP eluates showed enrichment of EGFR and known direct/indirect interactors
  - ✓ EGFR enrichment and phosphorylation was confirmed by Western blot analysis
  - ✓ Phospho-EGFR was also detected by MS (data not shown)

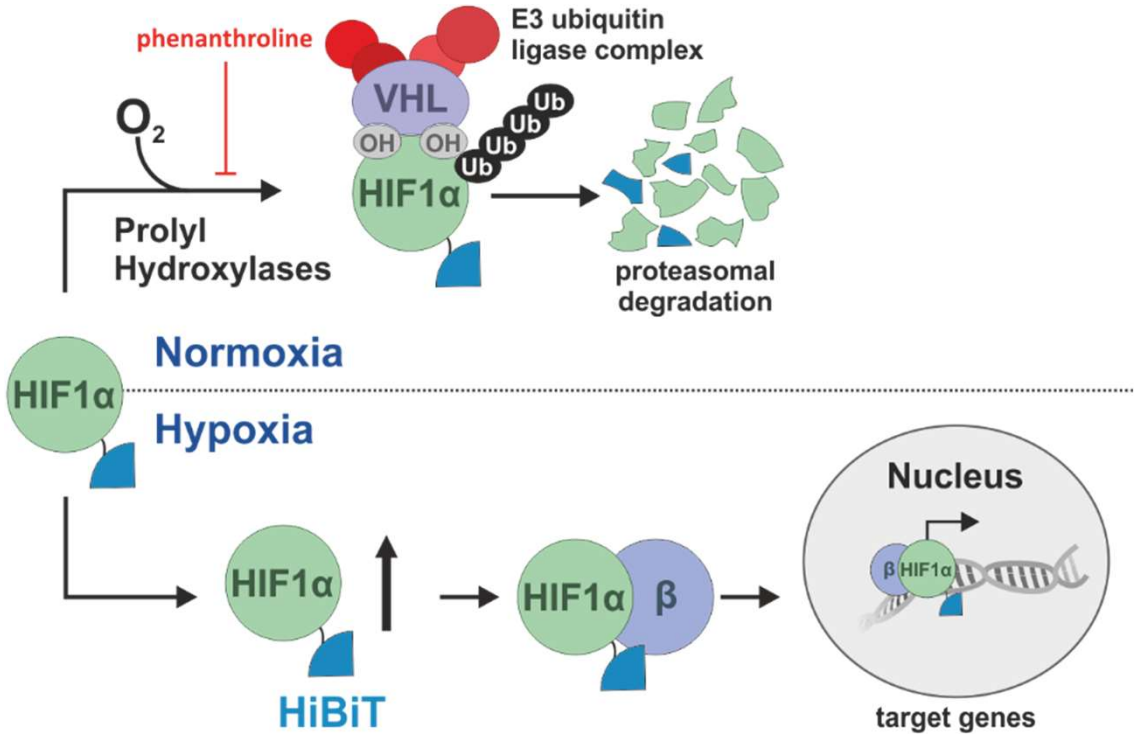
HiBiT Application Portfolio

One Bioluminescent Tag, Endless Possibilities



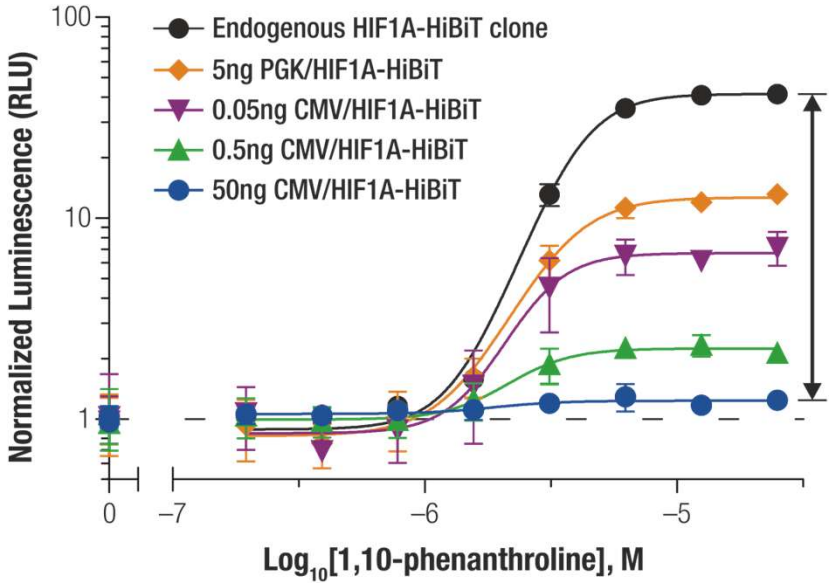
The HIF1α Pathway

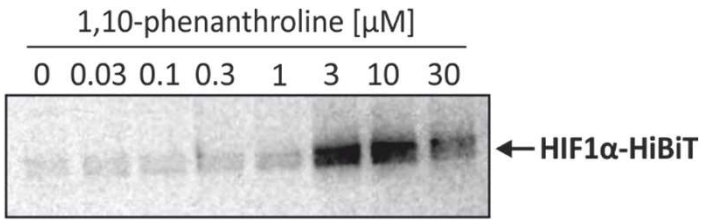
A Model System for Protein Stabilization



Stabilization of HIF1α

The Relevance of Expression Level Protein Stabilization

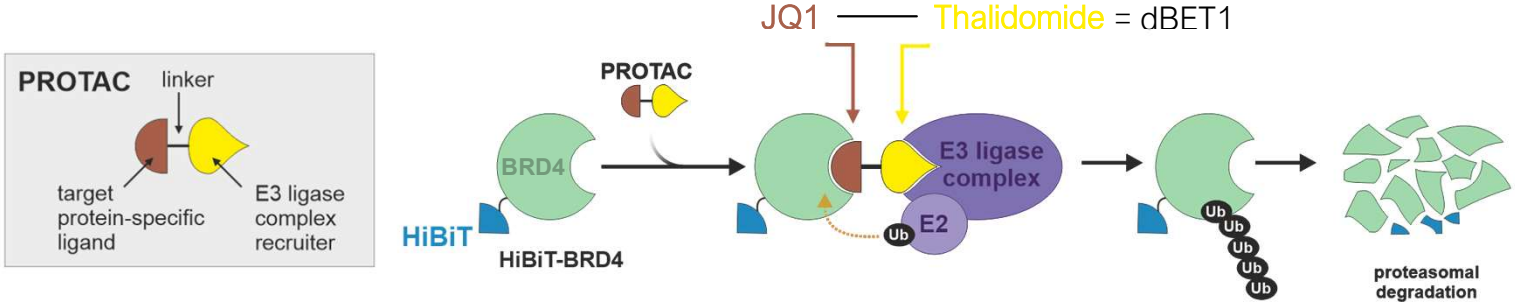


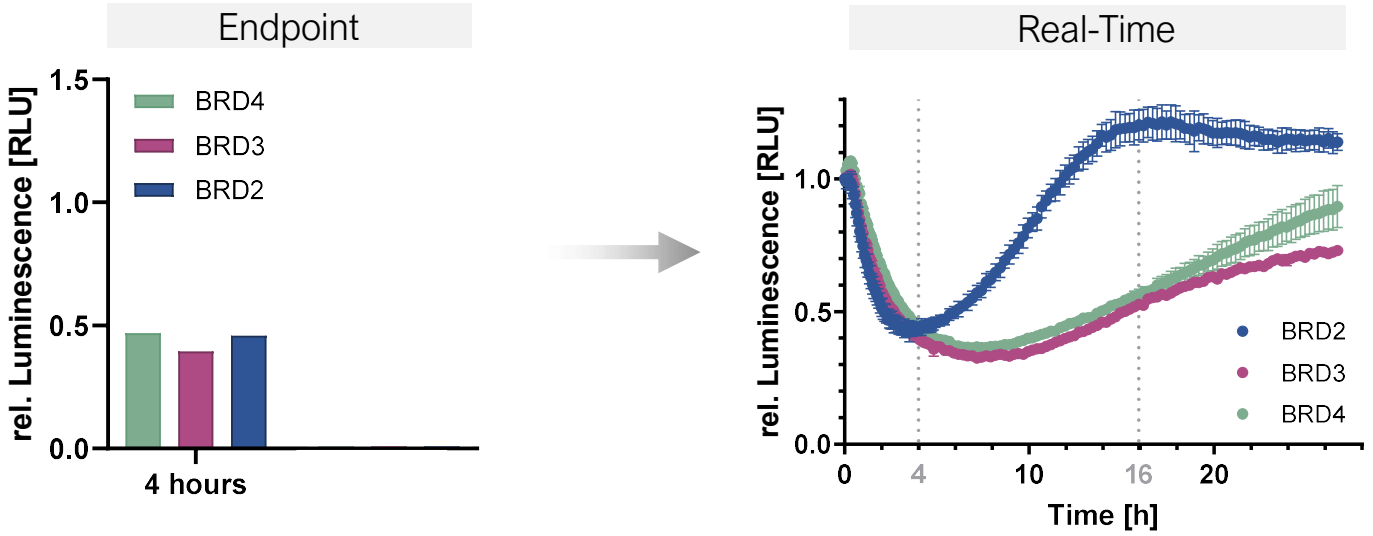


- High expression levels mute the biological response
- Endogenous expression yields highest assay window

Studying Targeted Protein Degradation

Proteolysis targeting chimeras (PROTACs)

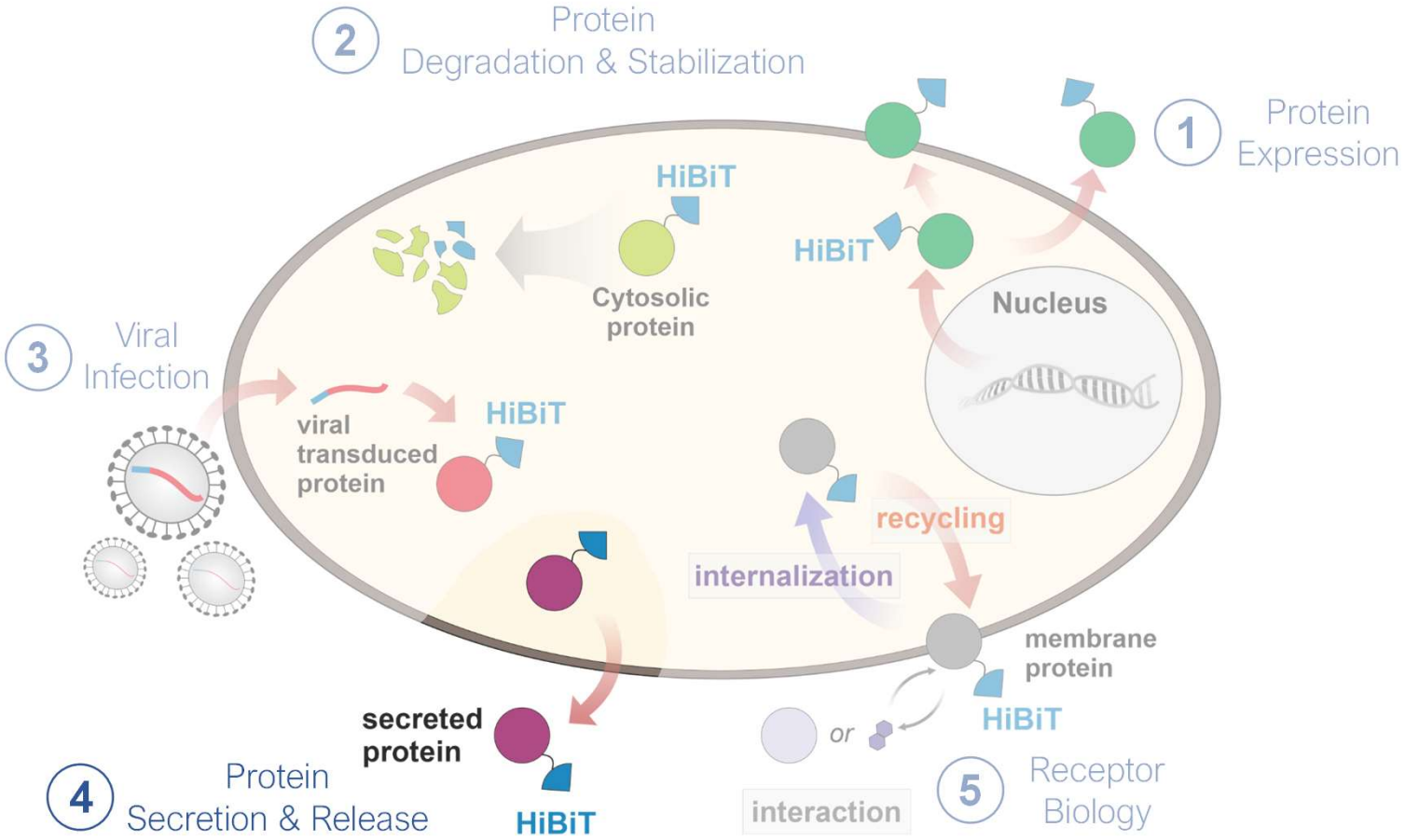




Riching et al. ACS Chem. Biol. 2018, 13, 9, 2758–2770.

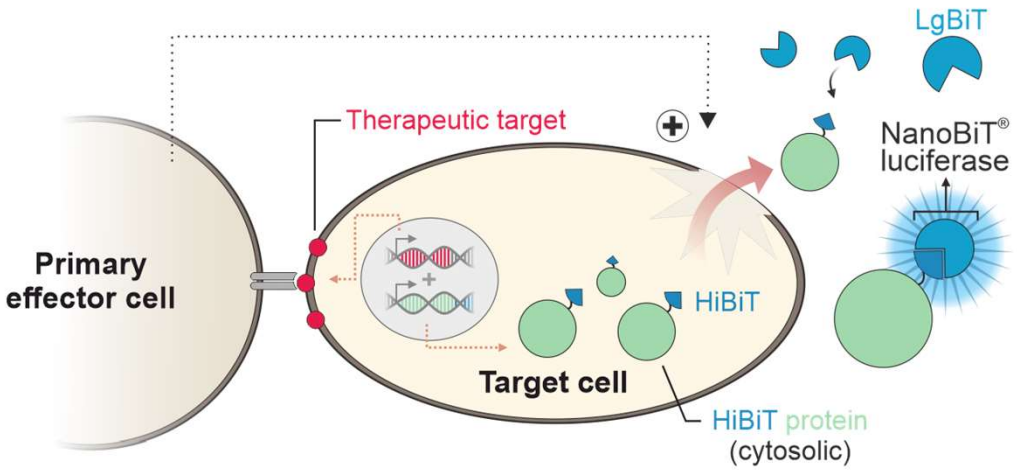
HiBiT Application Portfolio

One Bioluminescent Tag, Endless Possibilities



HiBiT Target Cell Killing Assay

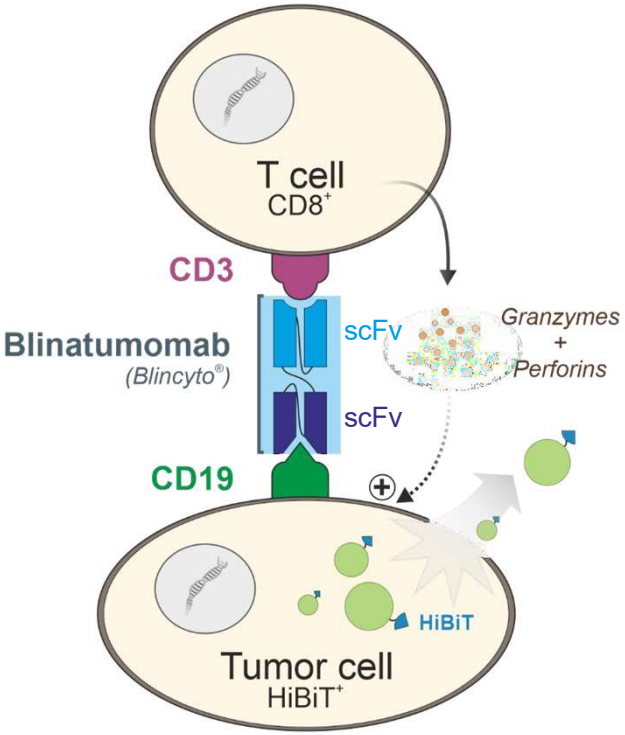
Measure death of a specific cell population within a mixed population of cells



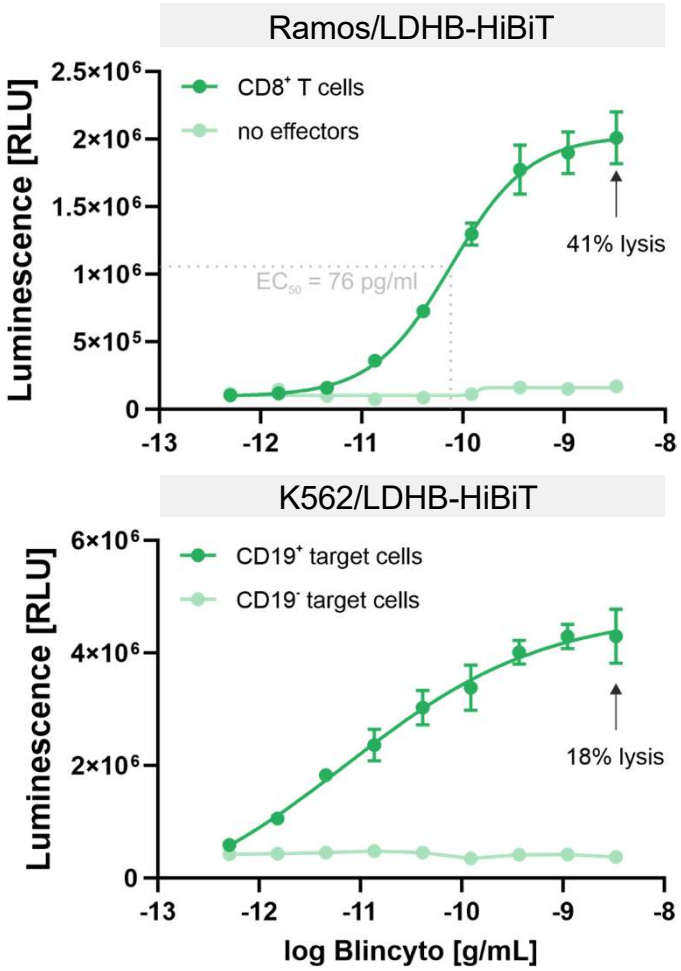
- Target cell with endogenous *or* ectopic expression of target and cytosolic HiBiT fusion protein
- Primary effector cells that mediate TCK and HiBiT release are added
- Released HiBiT is detected by LgBiT and NanoBiT® luciferase substrate addition
  - Endpoint or kinetic analysis possible

T Cell Dependent Cellular Cytotoxicity (TDCC)

Bispecific T-Cell Engager (BiTE)

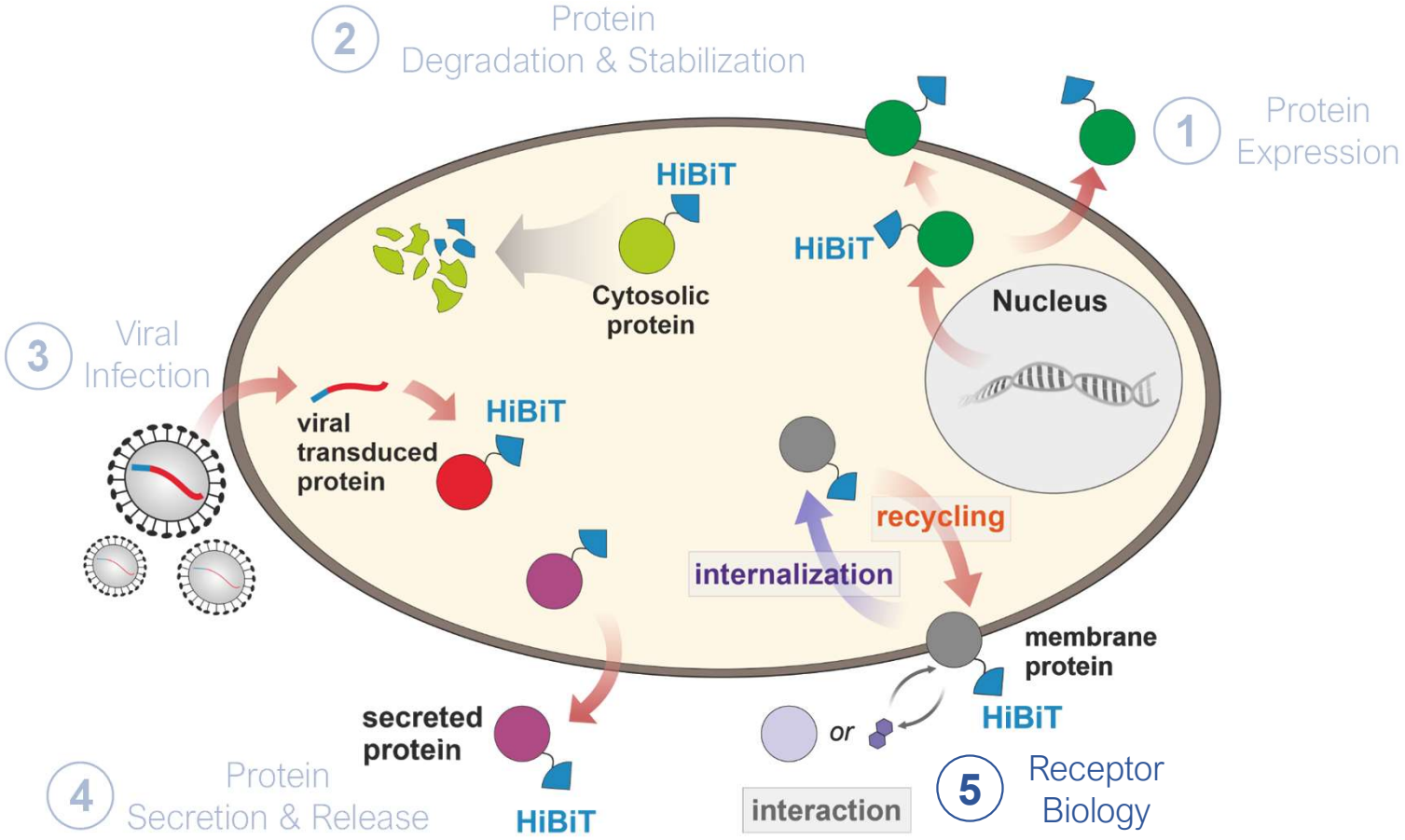


scFv: single-chain variable fragment



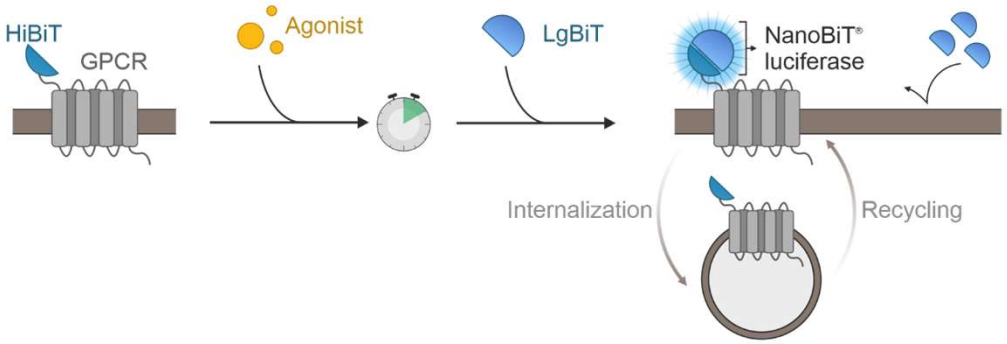
HiBiT Application Portfolio

One Bioluminescent Tag, Endless Possibilities



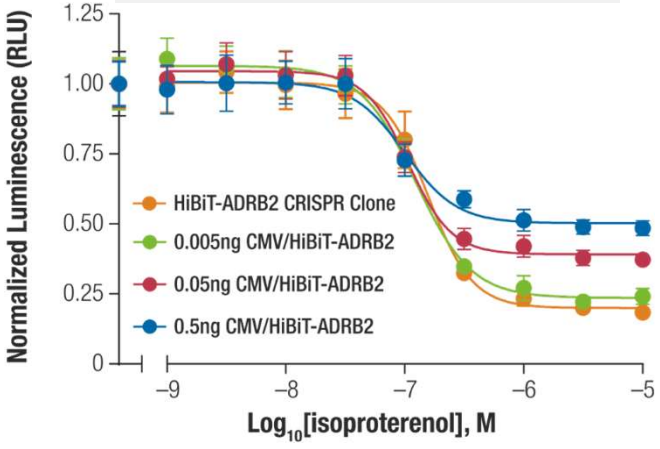
Study Receptor Internalization with HiBiT

GPCRs & RTKs

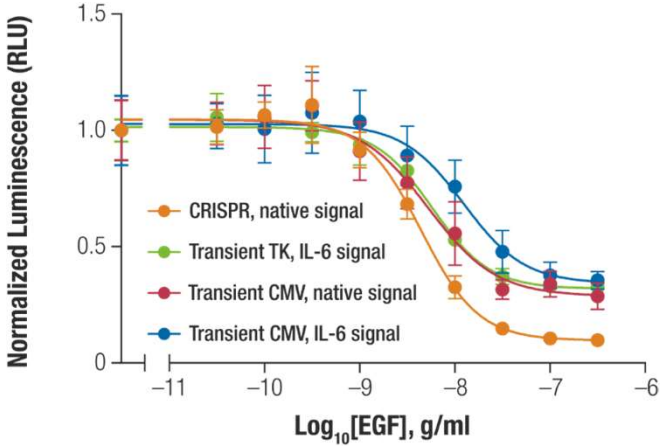


- Ectodomain of receptor tagged HiBiT
- Non-lytic detection with cell-impermeable LgBiT protein
- Measure ligand potency & internalization within minutes

HiBiT-ADBR2

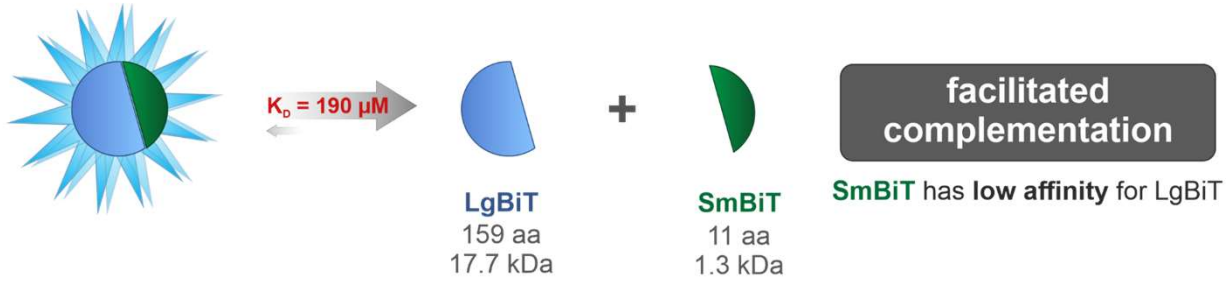


HiBiT-EGFR



NanoLuc® Binary Technology (NanoBiT®)

A Structural Complementation Reporter Designed for Biomolecular Interaction Studies



Complementation facilitated through …

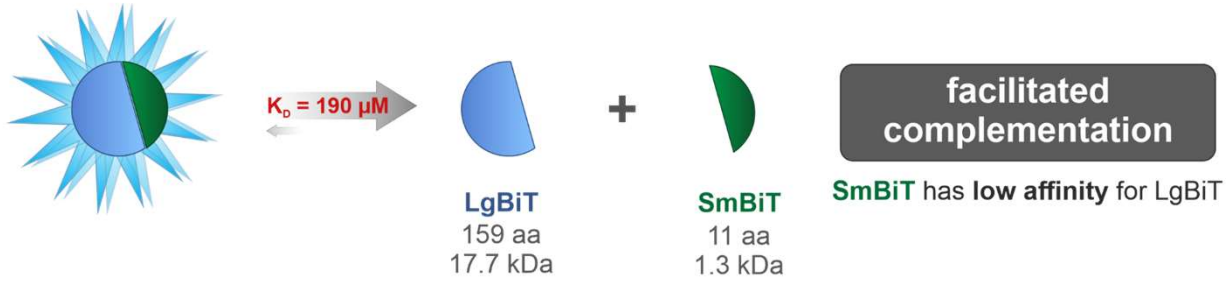
(direct) protein:protein interaction → NanoBiT® PPI System

Dixon *et al*. 2016, *ACS Chemical Biology*

Analyte Quantification

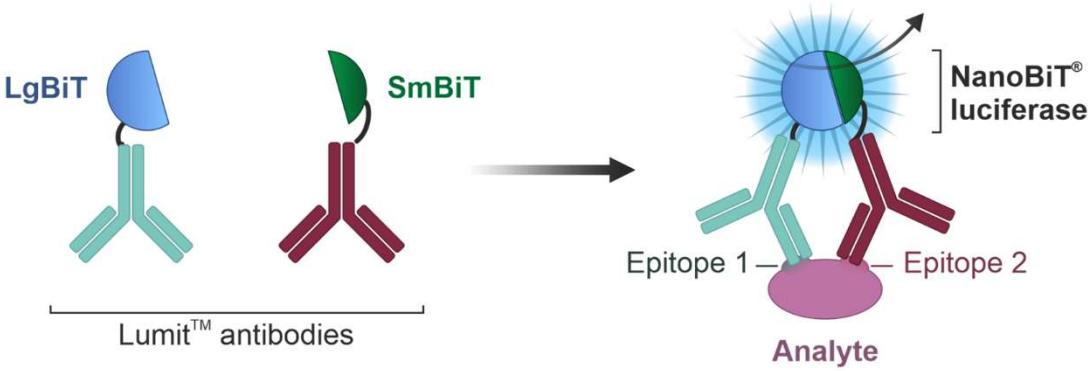
NanoLuc® Binary Technology (NanoBiT®)

A Structural Complementation Reporter Designed for Biomolecular Interaction Studies



Complementation facilitated through … (indirect) Ab:Ab "interaction" → Lumit™ Immunoassays

Hwang *et al*. 2020, *Commun Biol.*

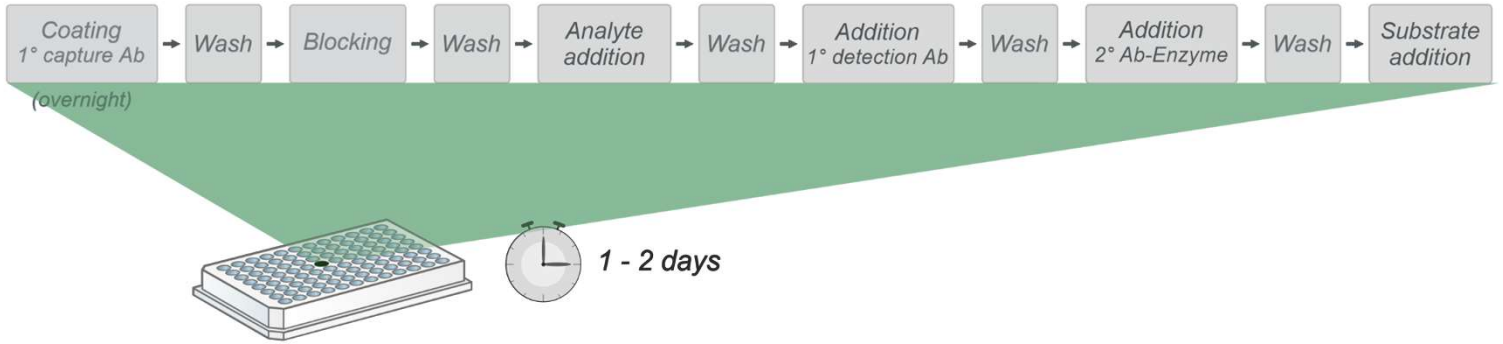


Analyte Quantification

Lumit Immunoassays

The Powerful Alternative to Conventional Immunoassay Approaches

Traditional ELISA Workflow



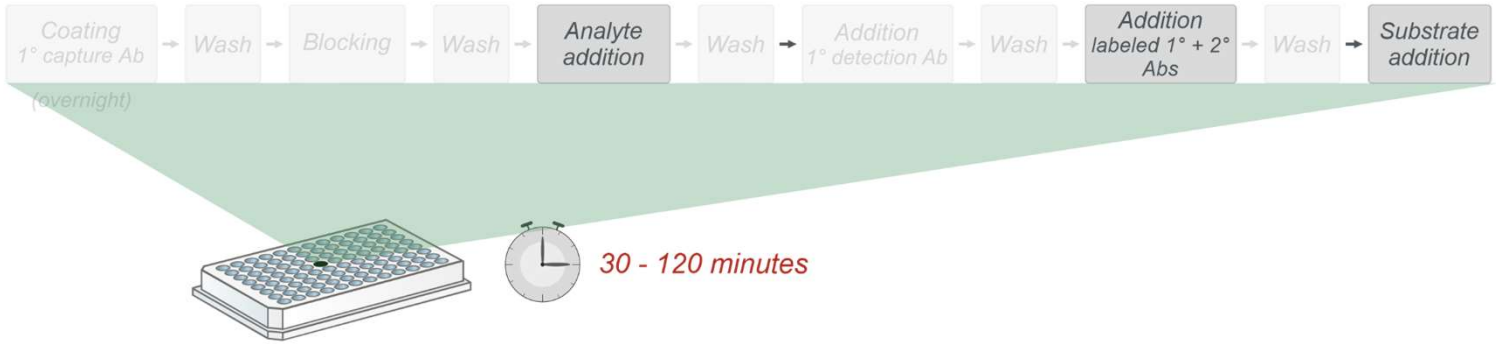
- Traditional ELISA is a heterogenous multistep process involving several wash / incubation steps

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

Lumit Immunoassays

The Powerful Alternative to Conventional Immunoassay Approaches

Lumit™ Immunoassay Workflow



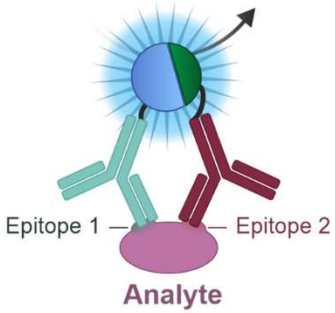
- Traditional ELISA is a heterogenous multistep process involving several wash / incubation steps
- Lumit™ Immunoassays
  - ✓ Easy workflow with short assay time (30 – 120 min)
  - ✓ High sensitivity (low number of cells)
  - ✓ Broad dynamic range (3 – 4 logs)
  - ✓ Flexible formats (96- or 384-well)
  - ✓ Homogenous and HTS compatible

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

Lumit Immunoassays

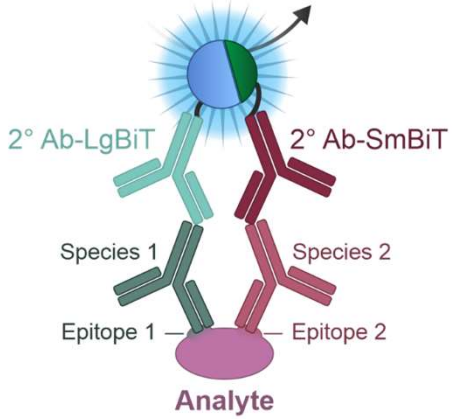
Different Formats for Maximum Flexibility

Direct



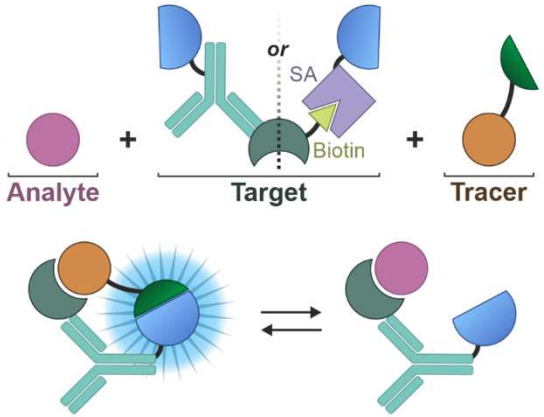
- Requires labeling of 1°Abs
- Validated for cytokines, peptide hormones, …
- *Ready-to-use* assays for
  - ✓ IL1-β, IFN-γ, IL-2, IL-6, IL-10, IL-4, IL-18, TNF-α, VEGF, insulin, glucagon, HMGB1, p24, Ki-67

Indirect



- Avoids labeling of 1°Abs
- Generic pre-labeled 2°Abs (different species available)
- Validated for intracellular PTMs, e.g. phosphorylation

Competitive

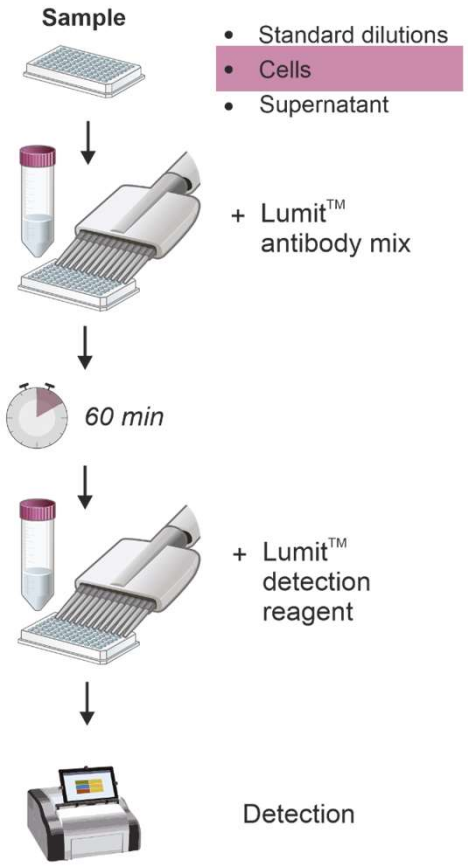


- Requires target and tracer labeling
- Establish competitive (antibody) binding assays
- *Ready-to-use* assays for
  - ✓ Lumit™ FcRn Binding Immunoassay
  - ✓ Lumit™ hFcγR Binding Immunoassays

    I , IIa (H131), IIa (R131), IIIa (V158), IIIa (F158)

Direct Lumit™ Cytokine Immunoassays

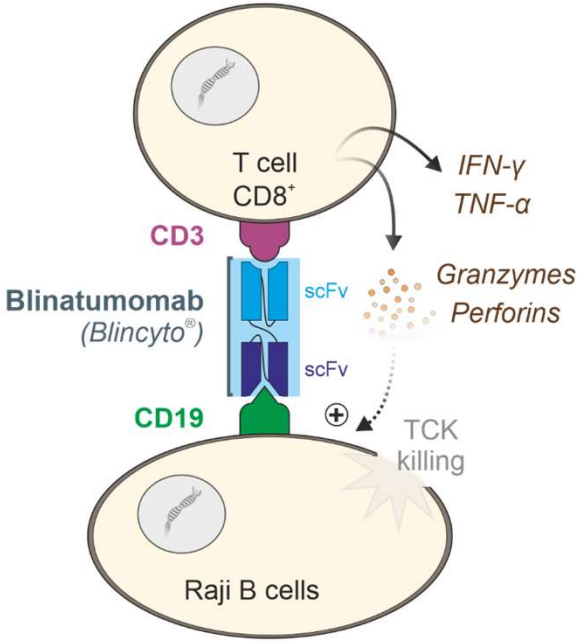
Flexible Protocol Options



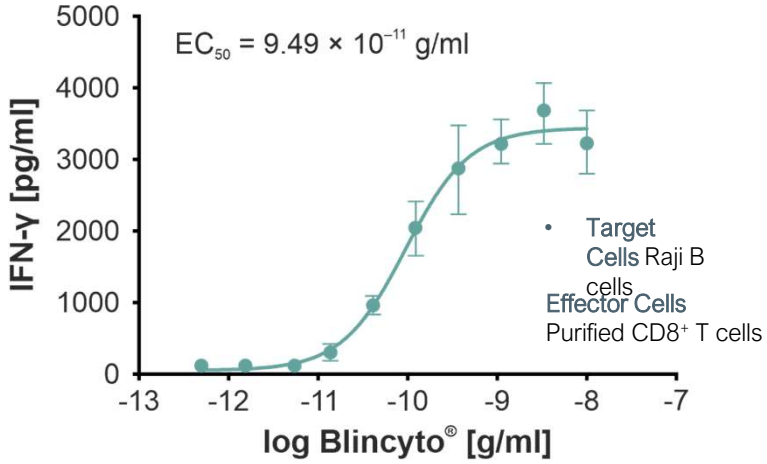
Direct Lumit™ Cytokine Immunoassays

Direct Addition (No Transfer) Protocol

BiTE-induced IFN-γ release from CD8⁺ T cells



Lumit™ IFN-γ (Human) Immunoassay



FACTS

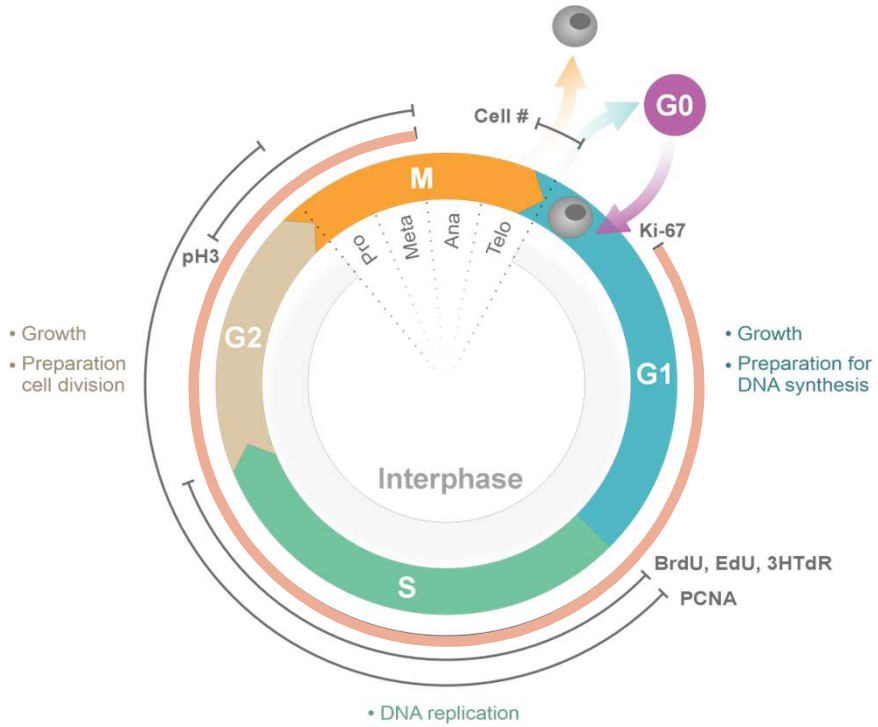
- Blincyto® binds to CD19 on cancer and CD3 on T cells
- T cell activation triggers cytokine release

BiTE: Bispecific T cell engager

scFv: single-chain variable fragment

Lumit™ hKi-67 Immunoassay for Cell Proliferation

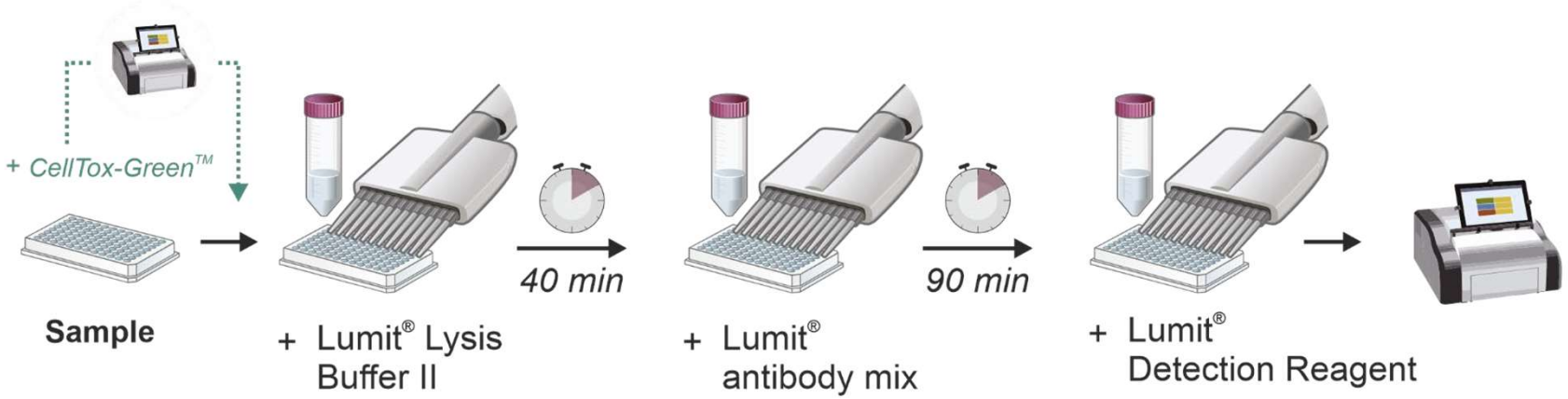
The proliferation marker Ki-67



- 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™ hKi-67 Immunoassay for Cell Proliferation

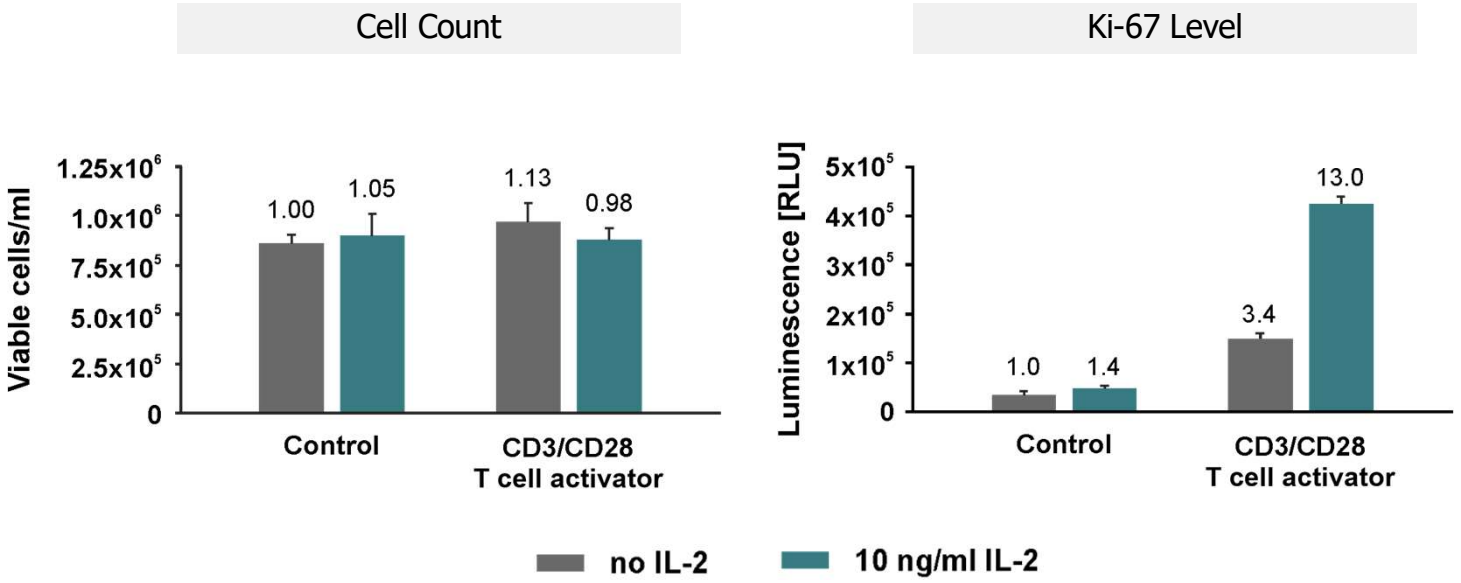
Workflow



- Completely homogeneous assay with no transfer or wash steps
- CellTox-Green™ Cytotoxicity Assay fluorescence readings for loss of membrane integrity (cell death) must be taken before initiating the Ki-67 assay protocol
- Antiproliferative activity is indicated by decreased Ki-67 levels without cell death

Lumit™ hKi-67 Immunoassay for Cell Proliferation

Ki-67 is an early indicator of cell proliferation

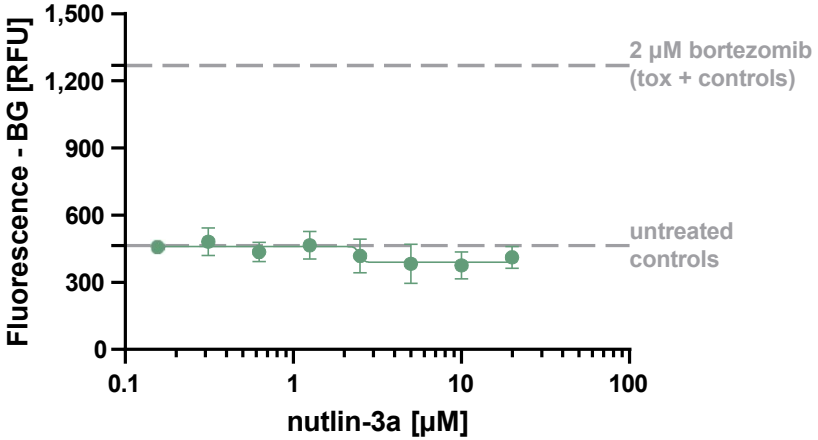


- Human CD8+ T cells (80,000/well) were treated with T cell activator (+/- IL-2) for 48 h
- Upregulation of Ki-67 is observed before T cell proliferation (which begins >72 h after activation; data not shown)

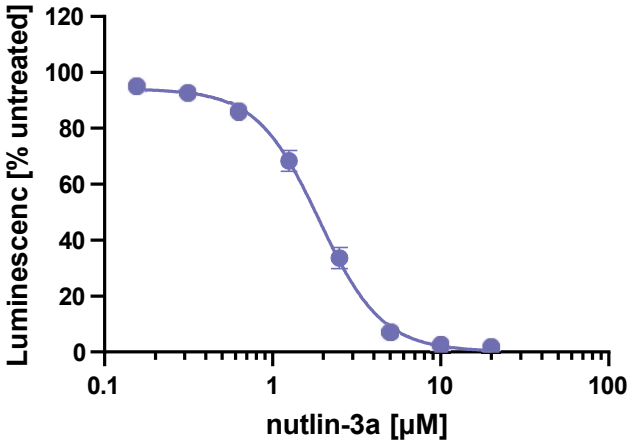
Lumit™ hKi-67 Immunoassay for Cell Proliferation

Ki-67 is an early indicator of cell proliferation

CellTox-Green™



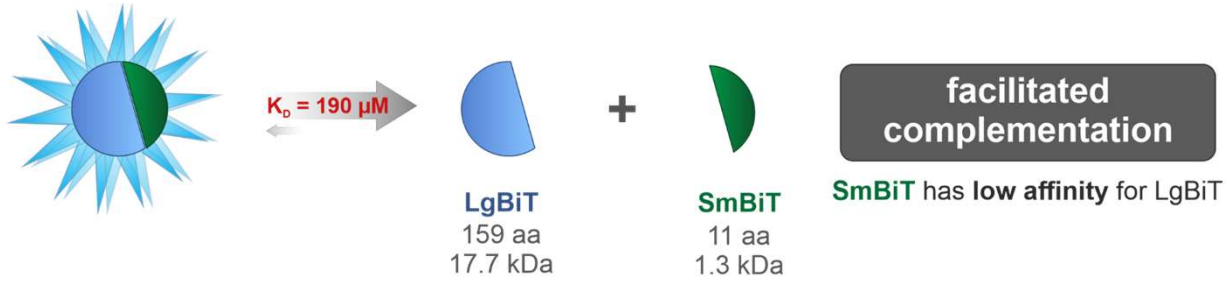
Lumit® hKi-67 Immunoassays



- HCT 116 cells (10,000/well) were treated with antiproliferative agent nutlin-3a for 48 hours
- Ki-67 expression was reduced in a dose-dependent manner without inducing cytotoxicity

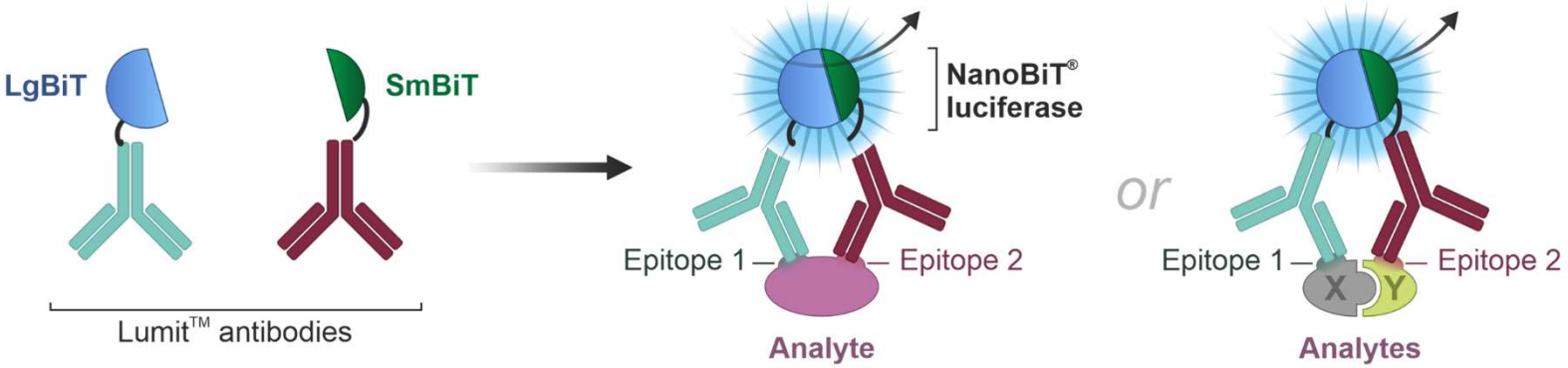
NanoLuc® Binary Technology (NanoBiT®)

A Structural Complementation Reporter Designed for Biomolecular Interaction Studies



Complementation facilitated through … (indirect) Ab:Ab "interaction" → NanoBiT® PPI System

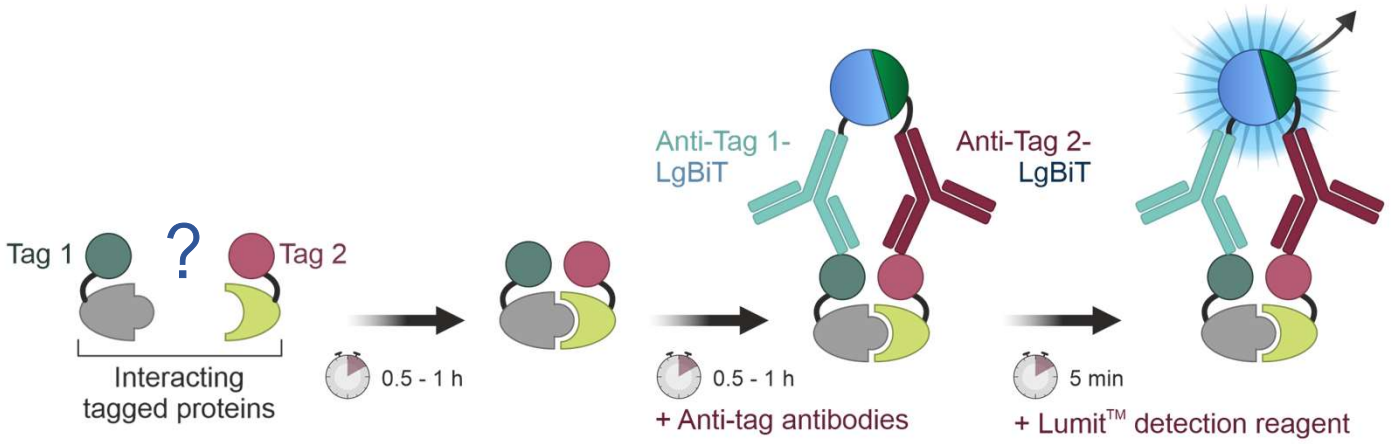
Hwang *et al*. 2020, *Commun Biol.*



Analyte Quantification

Lumit™ Anti-Tag Antibodies / Streptavidin

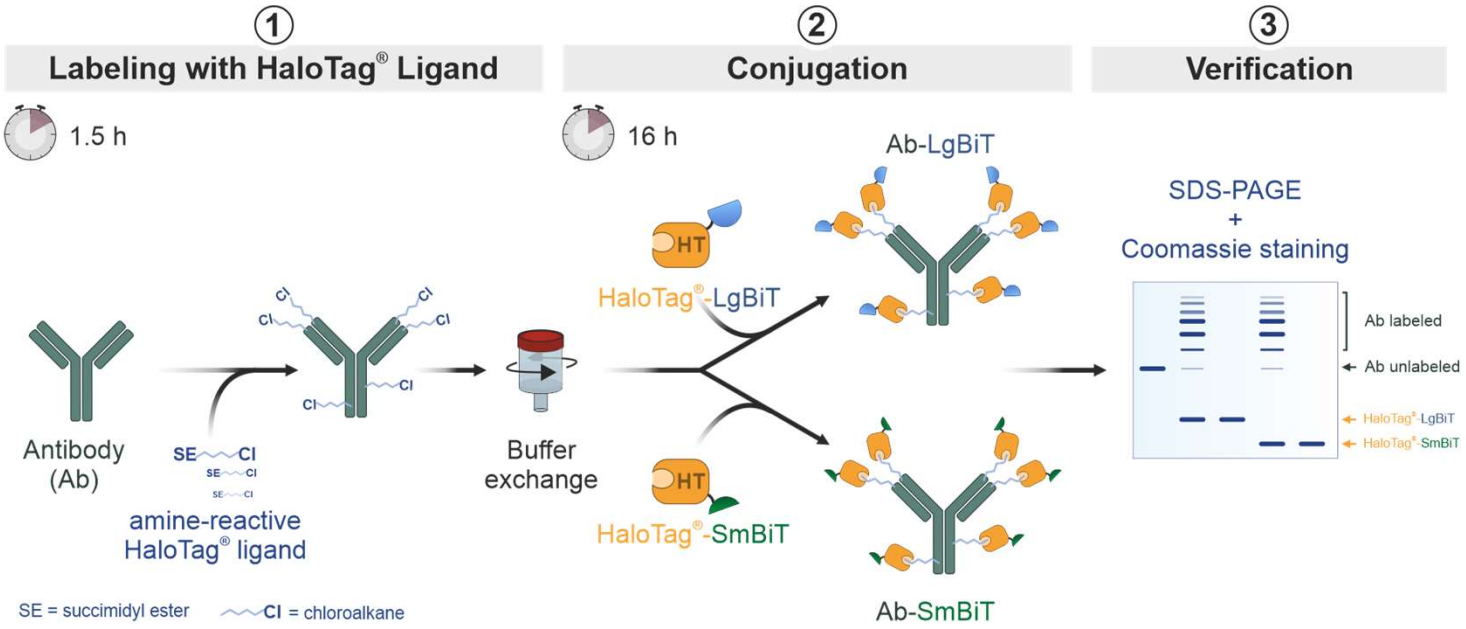
Detection of Protein:Protein Interactions in a Biochemical Assay



- His-tag detection
  - Anti-His-tag antibody-LgBiT
  - Anti-His-tag antibody-SmBiT
- GST-tag detection
  - Anti-GST-tag antibody-LgBiT
  - Anti-GST-tag antibody-SmBiT
- FLAG®-tag detection
  - Anti-FLAG®-tag antibody-LgBiT
  - Anti-FLAG®-tag antibody-SmBiT
- Human/Mouse/Rabbit Fc-tag detection
  - Anti-Human/Mouse/Rabbit IgG-LgBiT
  - Anti-Human/Mouse/Rabbit IgG-SmBiT
- Biotin/Avi-tag detection
  - Streptavidin-LgBiT
  - Streptavidin-SmBiT
- Self-labeling

Build-Your-Own Direct Lumit™ Immunoassay

Step 1: Labeling of Antibodies with the Lumit® Immunoassay Labeling Kit

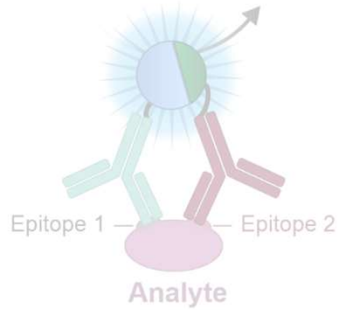
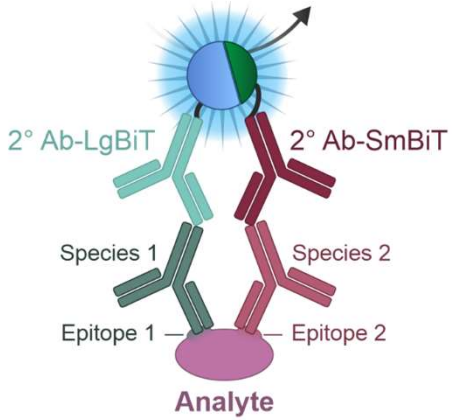
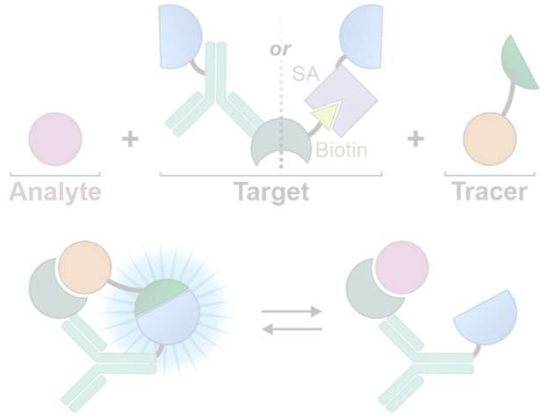


FACTS

- Easy and robust 2-day protocol
- Attachment is highly efficient (> 90%)
- Oriented BiT subunits for maximum activity
- Removal of unbound HT-BiTs is usually not required
- Clean-up can be easily performed using Magne® HaloTag® Beads

Lumit Immunoassays

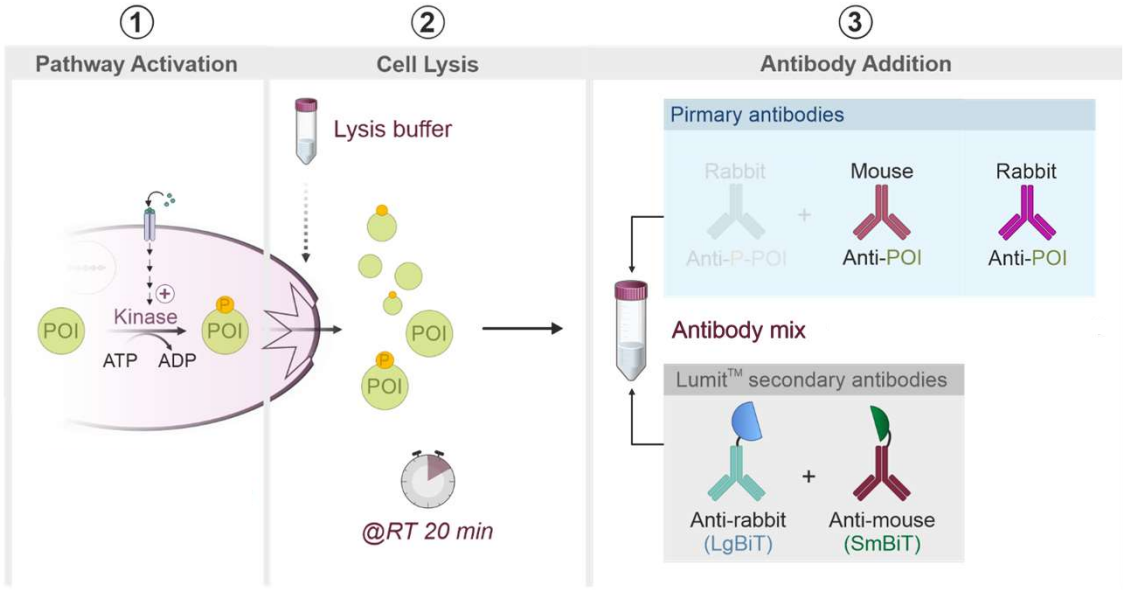
Different Formats for Maximum Flexibility

Direct	Indirect	Competitive
		
• Requires labeling of 1°Abs • Validated for cytokines, peptide hormones, … • *Ready-to-use* assays for ✓ IL1-β, IFN-γ, IL-2, IL-6, IL-10, IL-4, IL-18, TNF-α, VEGF, insulin, glucagon, HMGB1, p24, Ki-67	• Avoids labeling of 1°Abs • Generic pre-labeled 2°Abs (different species available) • Validated for intracellular PTMs, e.g. phosphorylation	• Requires target and tracer labeling • Establish competitive (antibody) binding assays • *Ready-to-use* assays for ✓ Lumit™ FcRn Binding Immunoassay ✓ Lumit™ hFcγR Binding Immunoassays I , IIa (H131), IIa (R131), IIIa (V158), IIIa (F158)

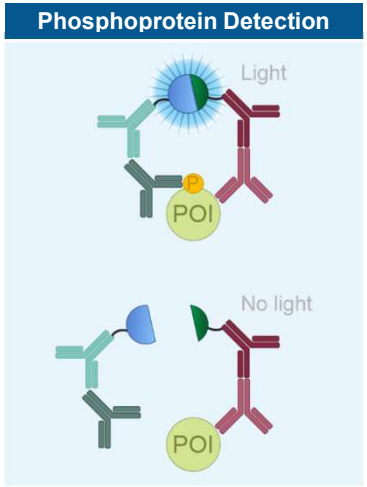
43

Lumit™ Immunoassay Cellular Systems

Study Cellular Signaling Events



Normalization to …



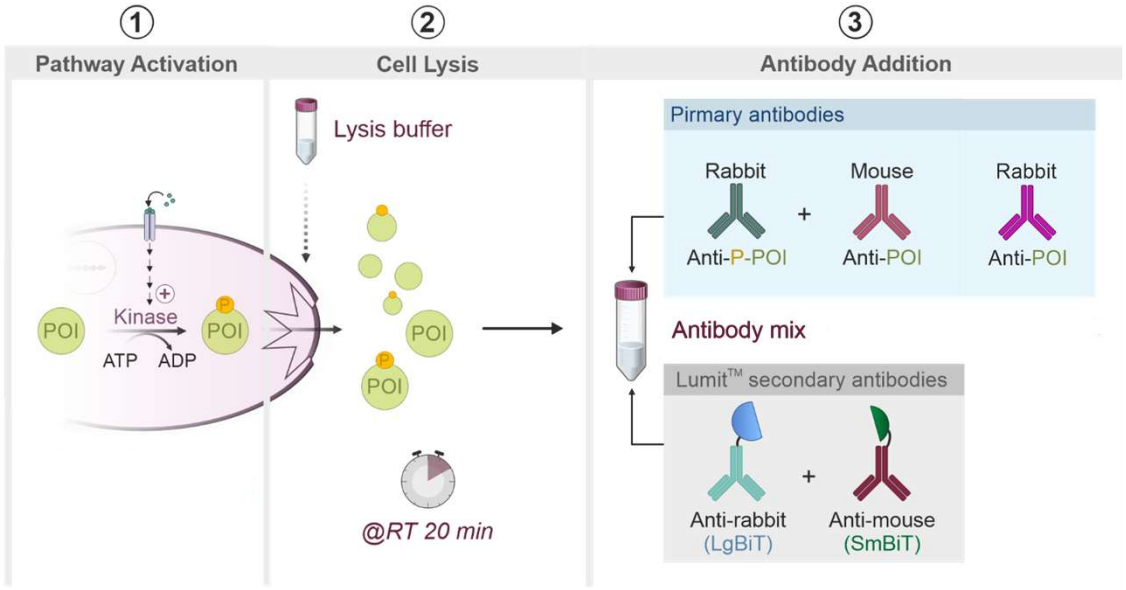
Available pre-labeled Lumit™ secondary antibodies:

- anti-rabbit (LgBiT) + anti-rabbit (SmBiT)
- anti-mouse (LgBiT) + anti-mouse (SmBiT)
- anti-goat (LgBiT) + anti-goat (SmBiT)

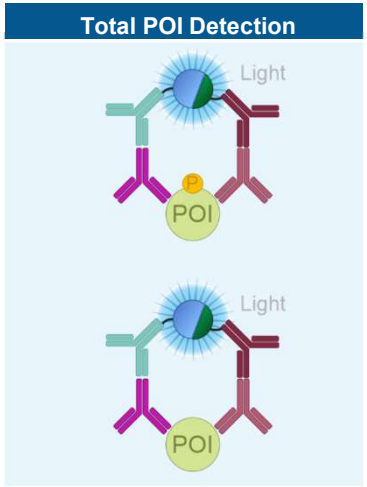
LCP: Live-cell protease

Lumit™ Immunoassay Cellular Systems

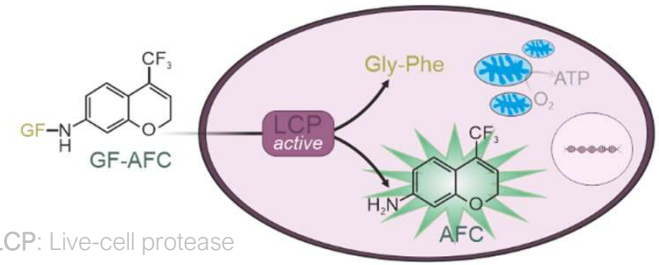
Study Cellular Signaling Events



Normalization to …



Normalization to number of viable cells



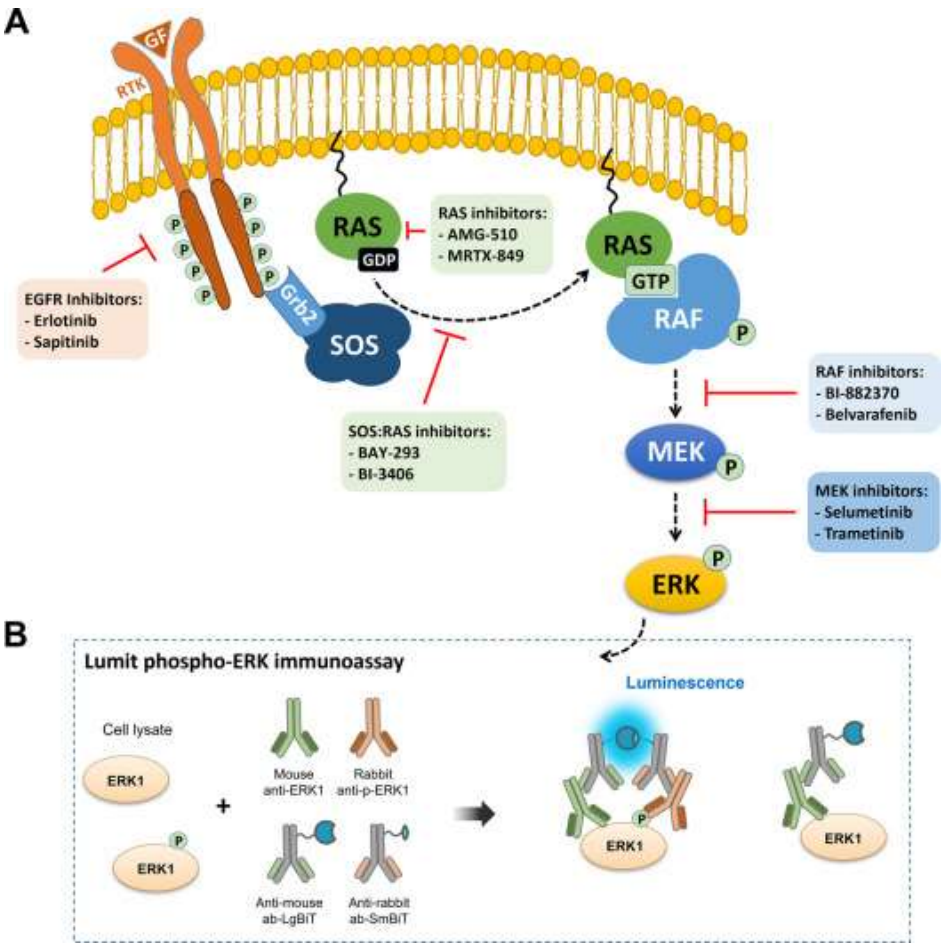
LCP: Live-cell protease

Available pre-labeled Lumit™ secondary antibodies:

- In live cells GF-AFC is processed into AFC by LCP
- Fluorescent AFC accumulates over time
- AFC signal correlates with viable cell number

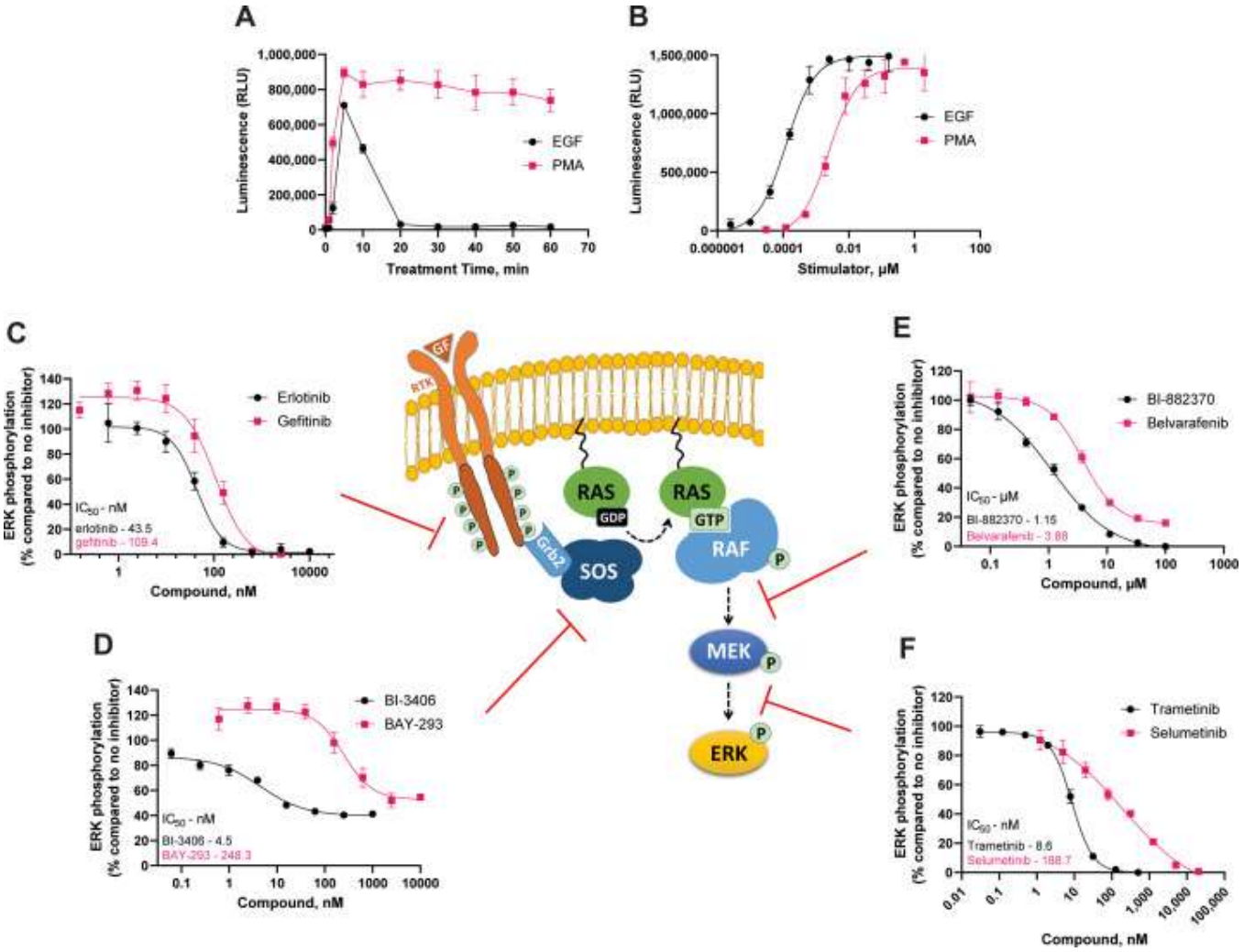
Analyzing RAS Signalling with Lumit Immunoassays

- KRAS is one of the most mutated oncogenes and targeting of its mutant forms has been difficult
- RAS/RAF/MEK/ERK pathway downstream of epidermal growth factor receptor (EGFR) activation
- EGFR activates son of sevenless 1 (SOS1) through the adapter protein GRB2
- SOS1 in turn, mediates the exchange of GDP for GTP within RAS which results in a phosphorylation cascade through the ERK-MAPK pathway, leading to phosphorylation of ERK
- The effect of different pathway inhibitors was monitored by detecting the ERK1 phosphorylation via Lumit indirect immunoassay



Swiatnicki et al. SLAS Discovery, 2022, Issue 4, Pages 249-257.

Inhibiting the RAS Pathway at Different Levels



Swiatnicki et al. SLAS Discovery, 2022, Issue 4, Pages 249-257.

Lumit™ Immunoassay Cellular Systems

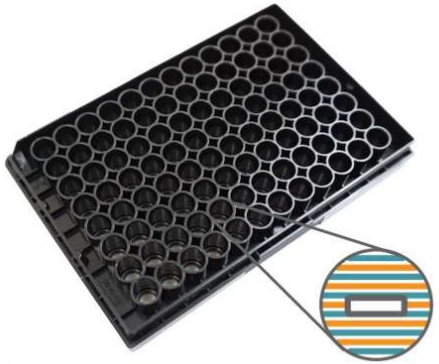
A Universal Immunoassay to Study Cellular Signaling

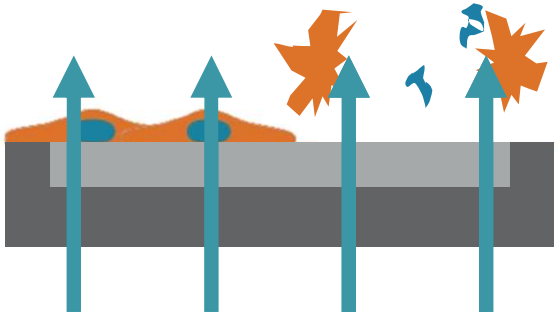
Validated with >20 phospho- and total proteins using 8 cell types, suggesting this universal immunoassay can be adapted for any pathway **with the appropriate antibodies**

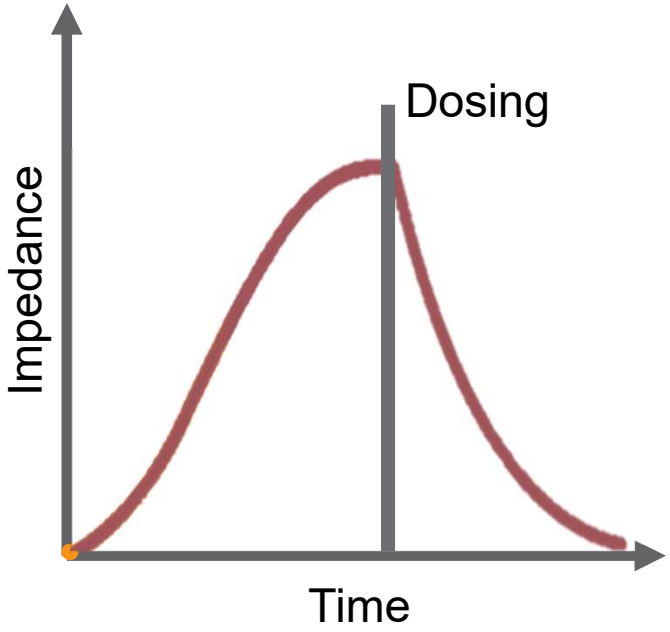
- AKT (phospho-Ser473 and total protein)
- BTK (phospho-Tyr223 and total protein)
- BCL6 (total protein)
- BRD4 (total protein)
- β -catenin (phospho-Thr41/Ser45 and total protein)
- CHK1 (phospho-Ser317)
- c-Jun (phospho-Ser63)
- cMET (phospho-Tyr1234/1235 and phospho-Tyr1349)
- CREB (phospho-Ser133 and total protein)
- EGFR (phospho-Tyr1068, phospho-Tyr1173 and total protein)
- Estrogen receptor (total protein)
- ERK1 (phospho-Thr202)
- GSK1-3 β(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 proteiin (phospho- Ser807/811 and phospho-Ser780)
- Ribosomall proteiin 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)

Impedance Assays Principle

- Measures how easily signal passes the electrode-cell interface
- Resistance increases as coverage and attachment increases
- Can detect:
  - Proliferation
  - Viability
  - Cell-cell coupling strength (barrier function)
  - Migration
  - Cell signaling

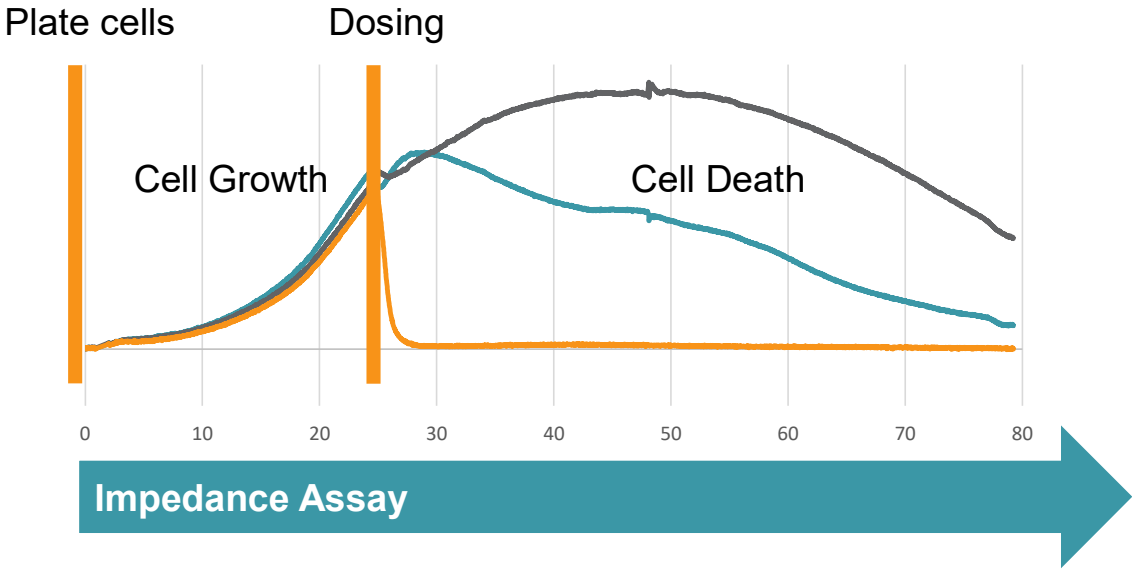




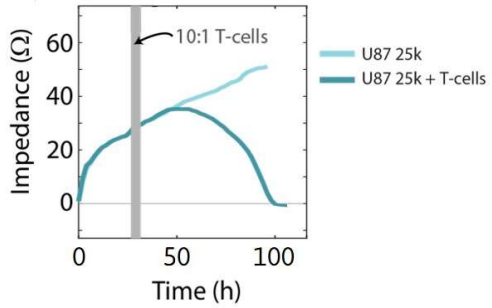


Impedance Assays vs. Traditional Assays

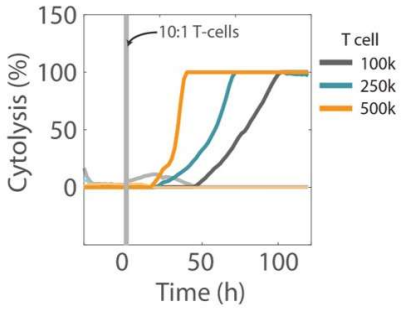
- Captures all stages of an experiment
  - Cell growth and death
  - Acute or chronic treatments
  - TEER measurements
- Hands-free data collection
  - Plate cells, add treatments, done
- Label-free
  - Measurement doesn't impact biology
  - No optimization of labels, dyes, or incubation times required
  - Multiplex with bioluminescent and fluorescent cell-based assays



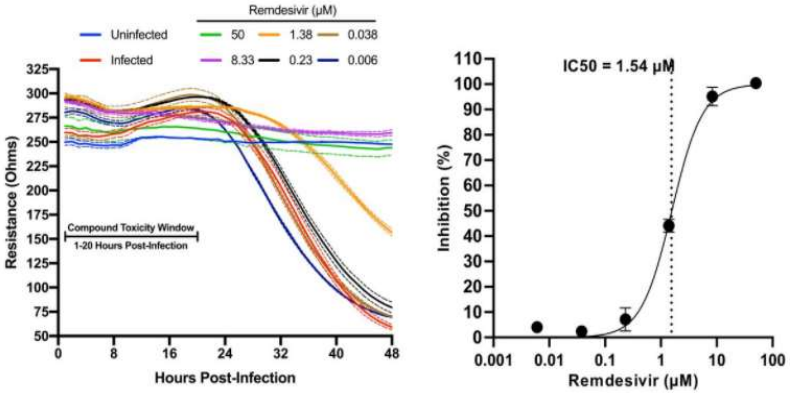
Impedance Measurement Applications



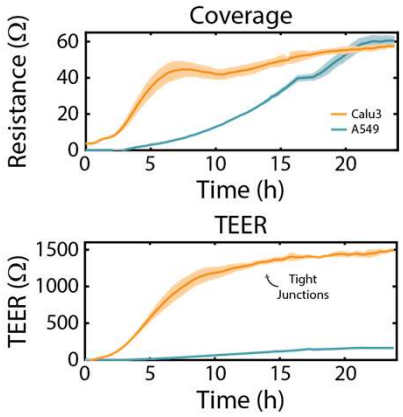
Immune cell kill assay

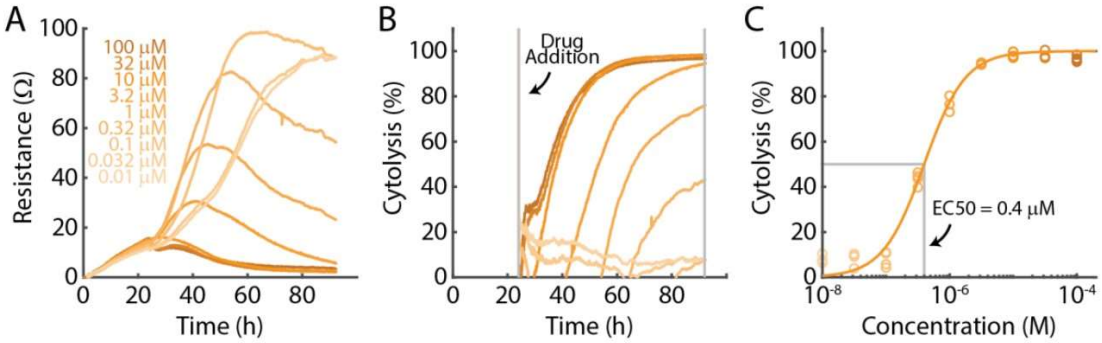


Kill curve



- **CAR-T therapy development**
- **Viral cytopathic effects**





SKOV3 cells treated with 9 different concentrations of doxorubicin

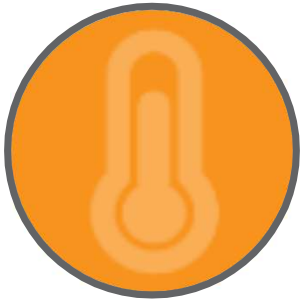
- **Transepithelial electrical resistance (TEER) changes**
- **Dose-response analysis**

Maestro Z Advantages

- One-button setup with barcode tracked plates
- Automated CO_2 and temperature control
- Automatic event tracking records door movements
- Multiplex measurements in the plates
- No computer needed while measuring
  - Only required for initiation and analysis
- Small footprint
- Mobile app for remote monitoring

Push-button Acquisition

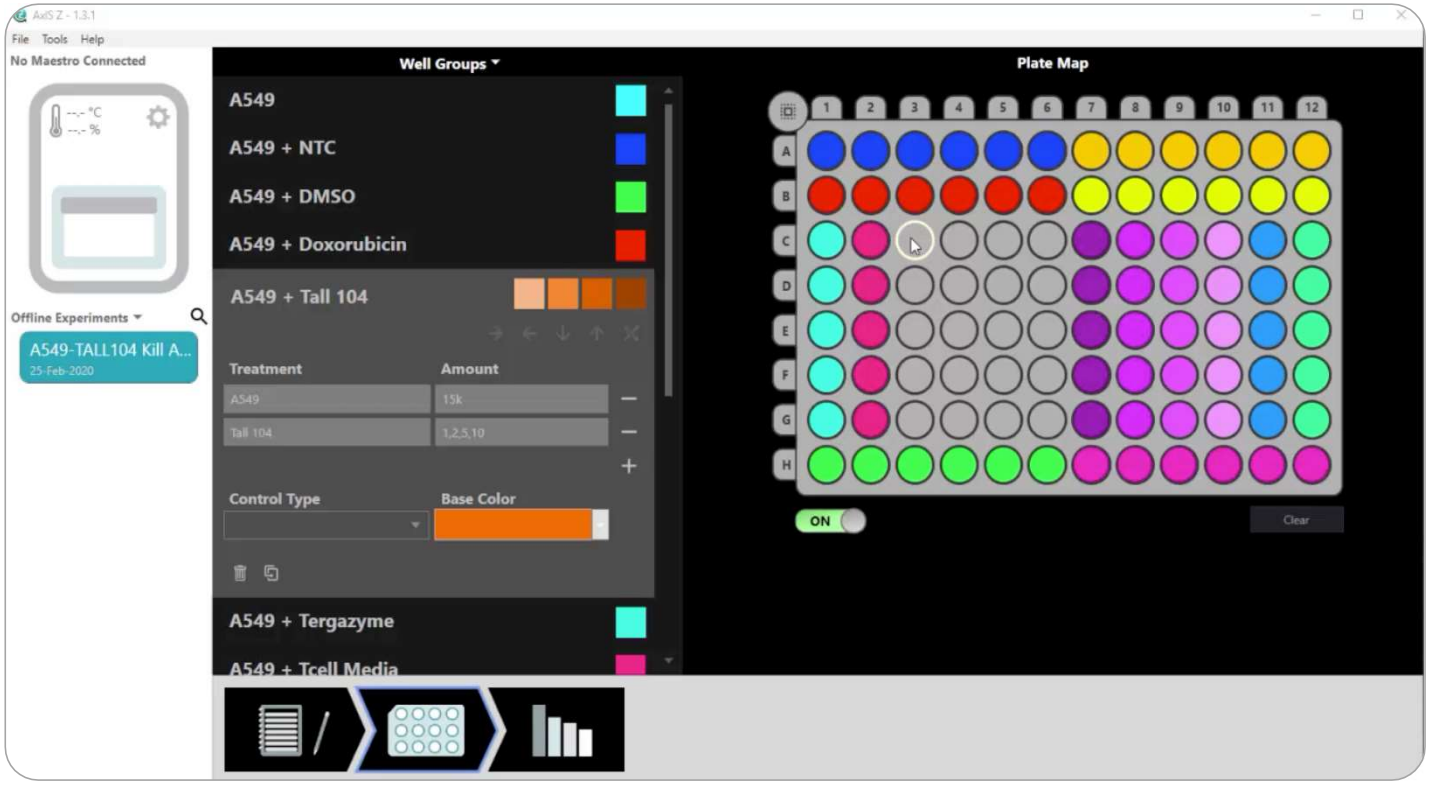
Barcode Tracking & Audit Trail



Automated Environmental Control

Label-free, continuous monitoring of cell behavior

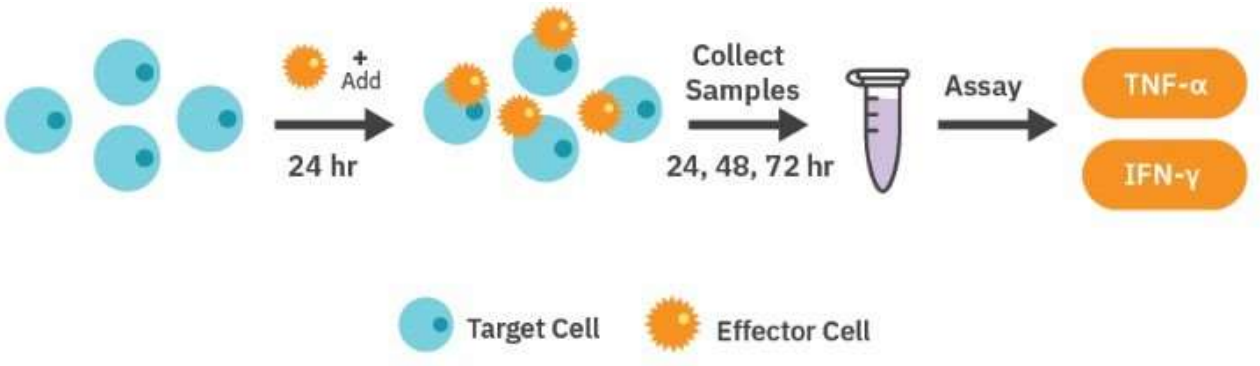
Quantitatively track cell proliferation, viability, and cytotoxicity



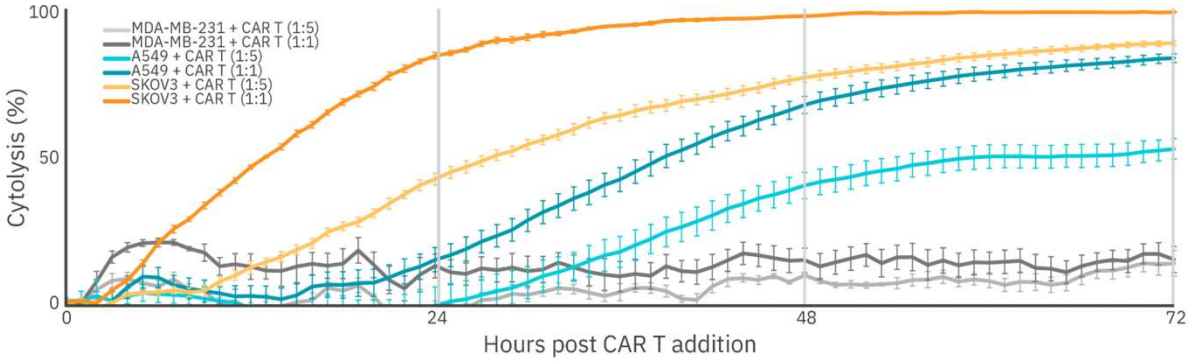
The only impedance assay that can be performed with a single-click

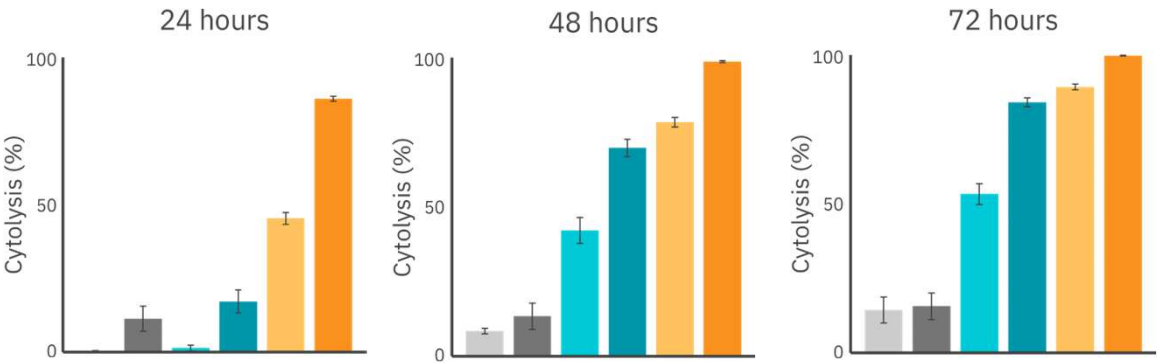
Combination of Impedance with Lumit Immunoassays for CAR-T development

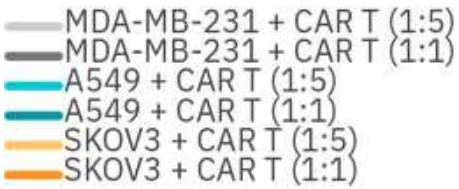
- Does tumor antigen density impact CAR-T Cell performance?
- HER2 CAR-T cells were cocultured with:
  - SKOV3 (high HER2 expression)
  - A549 (low HER2 expression)
  - MDA-MB-231 (no HER2 expression)
- CAR-T cell killing monitored by impedance, TNF-α & IFN-γß detected by Lumit Immunoassays



Combining Impedance with Bioluminescent Assays for CAR-T development

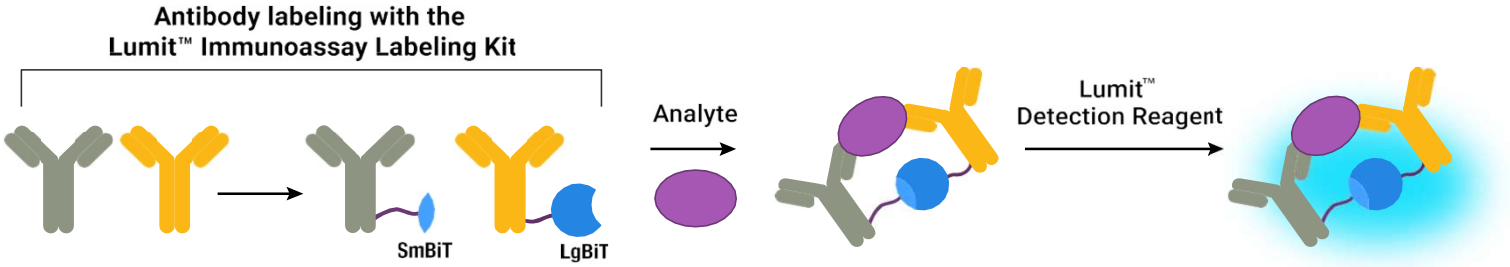




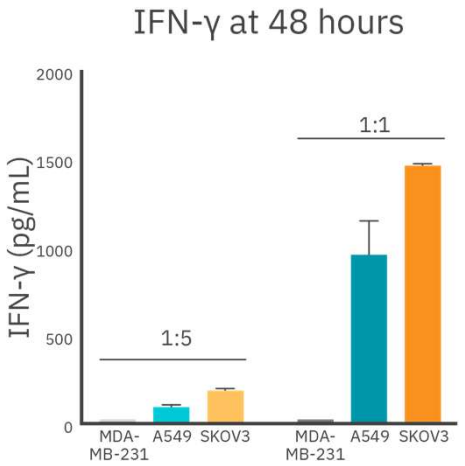


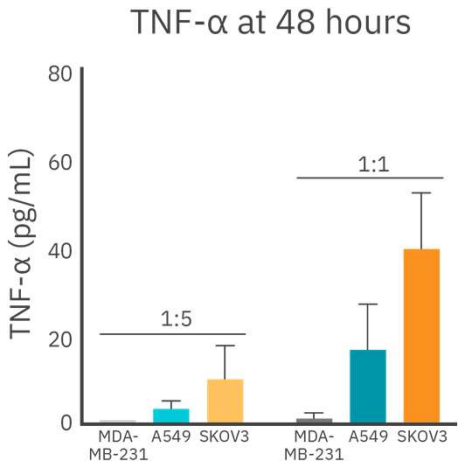
- CAR-T cell killing correlated with antigen expression levels
- Observed differences in cell killing change over time
- MDA-MB-231 cells showed 20% cytolysis due to nonspecific killing

Combining Impedance with Bioluminescent Assays for CAR-T development



- CAR T cells co-cultured with SKOV3 (high HER2) released 41.6% more IFN-γ compared to A549 (low HER2)
- CAR T cells co-cultured with SKOV3 released 80.5% more TNF-α compared to A549
- CAR T cells co-cultured with MDA-MB-231 (no HER2) did not release detectable TNF-α or IFN-γ





GloMax Galaxy Bioluminescent Imager

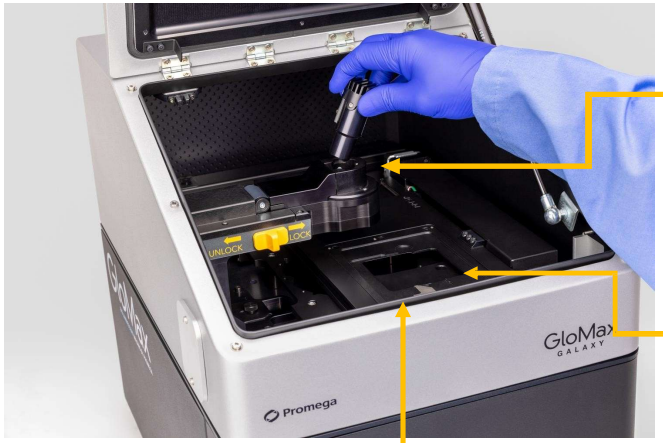
- Use NanoLuc® technologies to study rare events and analysis of mixed cell populations
- Study protein dynamics and cellular physiology
- Living & fixed cells & tissues
- Ideal for assay development

LUMINESCENCE	FLUORESCENCE	BRIGHTFIELD
Protein dynamics and localization	Cellular reference markers	Morphology

Affordable, Easy to use, Low-throughput

- Includes PC and monitor
- Compatible with slides, microchambers, dishes, and plates
- Motor-driven focusing and alignment
- 20X objective lens (10X overall magnification)
- Accessory: Environment Chamber (temperature, humidity, gas)

Instrument Overview



Fluorescence LED excitation arm

Stage with vessel holder

Objective (not shown in the photo)

- Easy excitation module exchange during an imaging session
- The system is supplied with 1 fluorescence excitation module (Blue 480/30)
- Additional modules can be purchased (DAPI, GFP, Texas Red, Cy3, Cy5, Janelia, MitoTracker Red, and more…)
- System designed to readily accept custom excitation modules

- Automated filter slide to change florescence emission filters via software
- 4-position slide, 3 pre-loaded
- Custom emission filters can also be ordered

Stagetop Incubator for Extended Live-Cell Imaging

- Incubator provides user control of the temperature, gas and humidity of the samples for long-term imaging
- Supplied from Japanese company Tokai Hit


Controller


Stagetop chamber

Tokai Hit Stage Holders for plates, slides, and dishes


Objective Lens Sensor


Power cables, and tubing for gas regulator

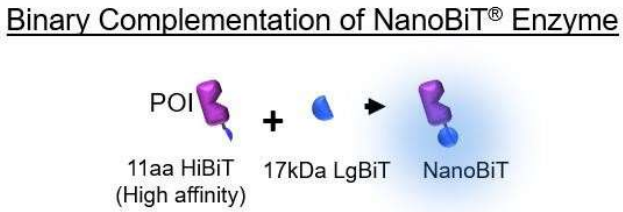

Water inlet tubing

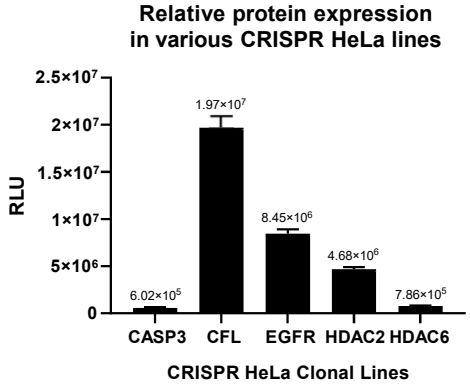

Tokai Hit Vessel Lids

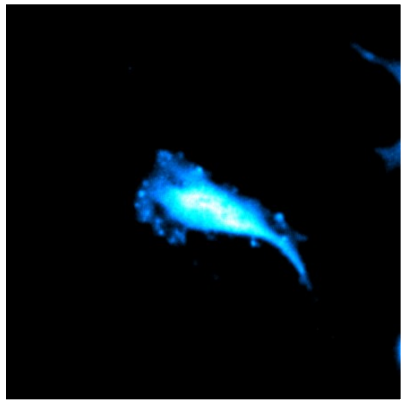
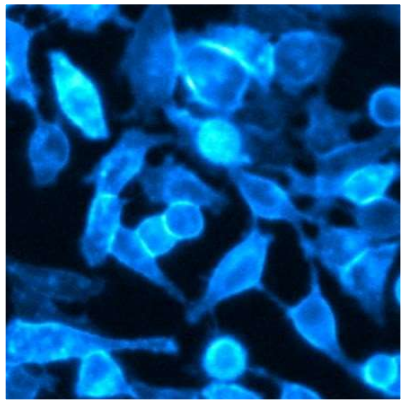
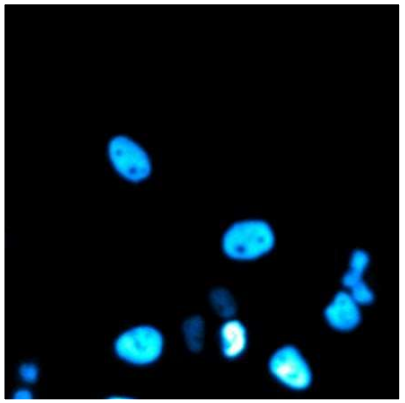
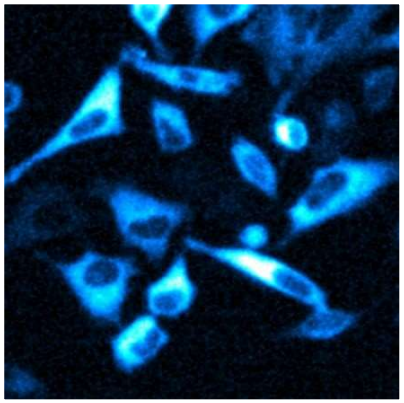
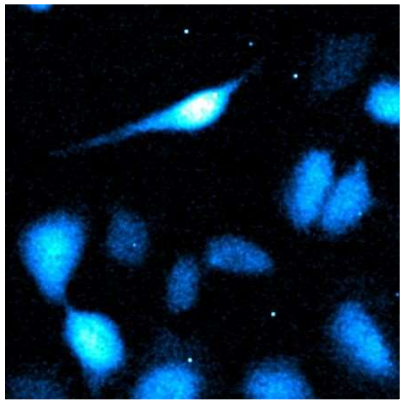
- Ideal vessels for live-cell imaging are Ibidi 8-well microchamber slides
- Due to longer exposure times, imaging a whole 96-well plate plate can take very long

Imaging Low Abundance Endogeneous Proteins

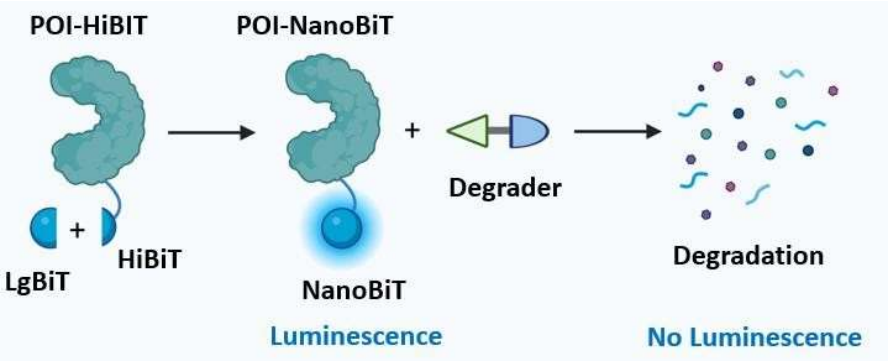
- HiBiT inserted to genomic locus via CRISPR/Cas9 in HeLa cells
- LgBiT expressed ectopically



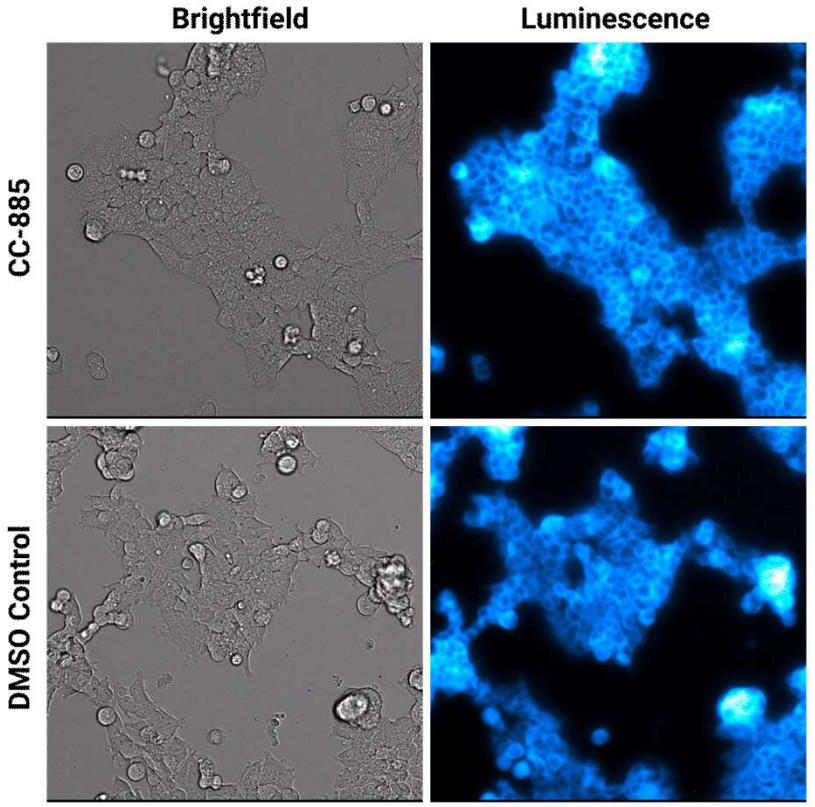


Cofilin	EGFR	HDAC2	HDAC6	CASP3
				
1-minute exposure	1-minute exposure	1-minute exposure	Low Expression ~3-minute exposure	Very Low expression ~5-minute exposure

Targeted Protein Degradation of Endogeneous GSPT1

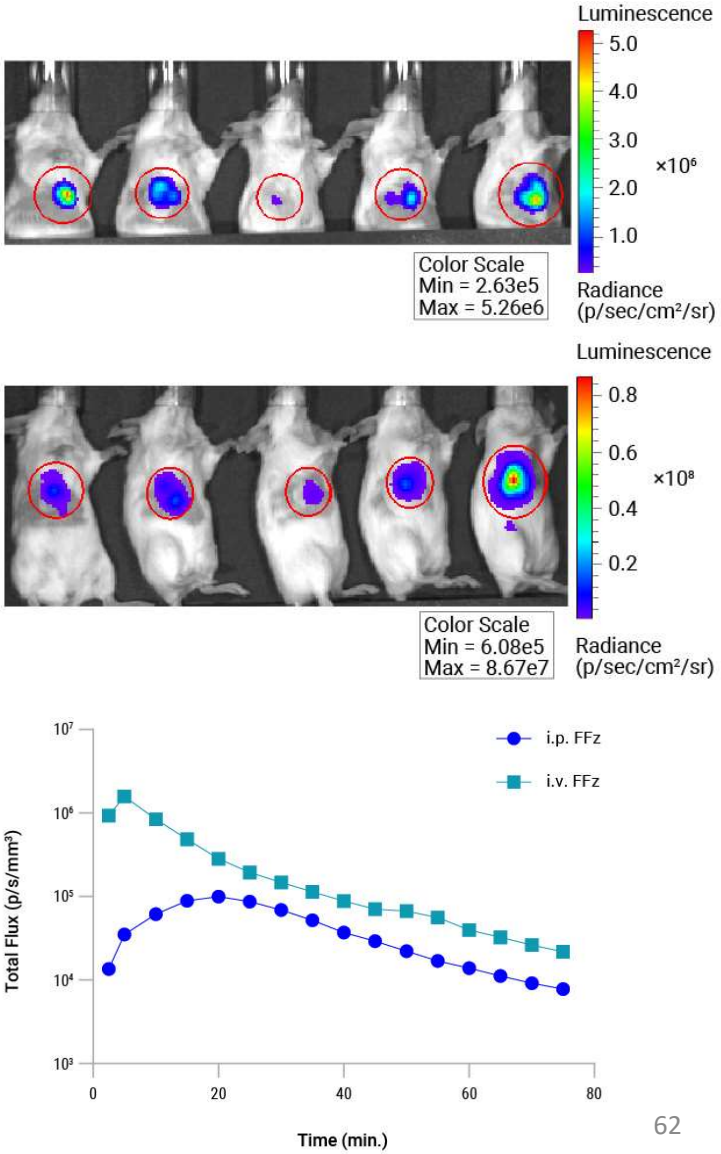


- HEK293 cells expressing endogenous HiBiT-tagged GSPT1 and stably expressing LgBiT were treated with CC-885 degrader or DMSO control treatment.
- Assayed with Nano-Glo® Vivazine Live Cell Substrate and imaged over 5 hours in stagetop incubator.
- Molecular-glue degrader, CC-885, facilitates targeted protein degradation of GSPT1, and acts as an anti- tumor agent.



Imaging NanoLuc In Vivo

- Nano-Glo Fluorofurimazine has increased aqueous solubility and allows increased substrate delivery
- Brighter in vivo signal and increased signal stability
- Greater flexibility in delivery options – intraperitoneal vs intravenous injection
- NanoLuc‘s small size makes it easy to pack into viral genomes and track virus tissue penetration for gene therapy or infectious disease applications



High Quality Cell Culture Media and Sera

- German company established in 2013
- Specialises on the production of high quality sera and cell culture media and reagents
- Possibility of custom manufacturing from 20 liters

Sera
- FBS
- Other bovine and animal sera
- Human sera

Cell Culture Media
- Classic liquid media
- Classic powdered media
- Special culture media
- Cryopreservation

Cell Culture reagents
- Supplements and additives
- Antibiotics
- BSA
- Trypsin
- Cell separation

Balanced Salt Solutions
- Liquid buffers
- Powdered buffers

Diagnostics
- Virology media
- Cytogenetics

High Quality Sera for Cell Culture

FBS Standard	FBS Advanced	FBS Xtra
Natural FBS	Slightly reduced raw FBS content	Reduced raw serum content
Low endotoxin	Cost-efficient with defined additives	Chemically defined additives
Consistent quality	Minimal lot-to-lot variations	Reduced lot-to-lot variations
Pricing highly dependant on raw FBS price fluctuations	No further batch testing necessary	More sustainable ingredients
Suitable for all cell types	Best for tumor cells & less demanding cell types	Best for tumor cells & fast-growing cell types

High Quality Sera for Cell Culture

CAPRICORN SCIENTIFIC

Until end of 2024, order FBS Minis for the price of standard 500ml bottles.

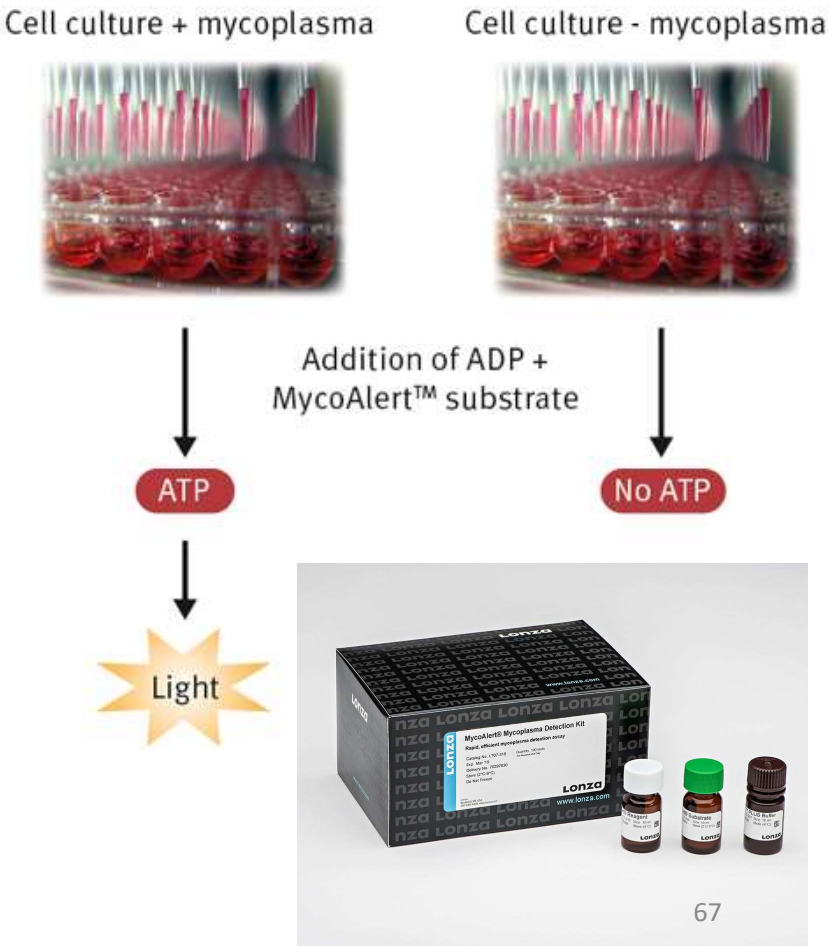
Primary Cells, Stem Cells and Media

Lonza

- Primary cells - over 150 human and animal cell types available
- Clonetics media a growth factors for wide spectrum of primary cells
- Stem cells together with media
- Blood and immune cells from vast collection of donors and sources
  - Specialized X-Vivo™ media

Mycoplasma Testing

- Widespread contamination in a variety of cell culture systems
- Size below 1 µm, hardly visible in optical microscope
- BL MycoAlert™ kit utilizes the Mycoplasma enzymes to convert ADP to ATP
- Converted ATP is consumed by firefly luciferase to produce bioluminiscence in case of contamination
- Requires only 100 µl of centrifuged medium for the assay



GloMax Plate Readers – Configurations

GloMax® Navigator

96-well

✓ Luminescence

GloMax® Explorer

6-, 12-, 24-, 48-, 96- and 384-well

✓ Heating
✓ Shaking
✓ Luminescence
✓ Fluorescence

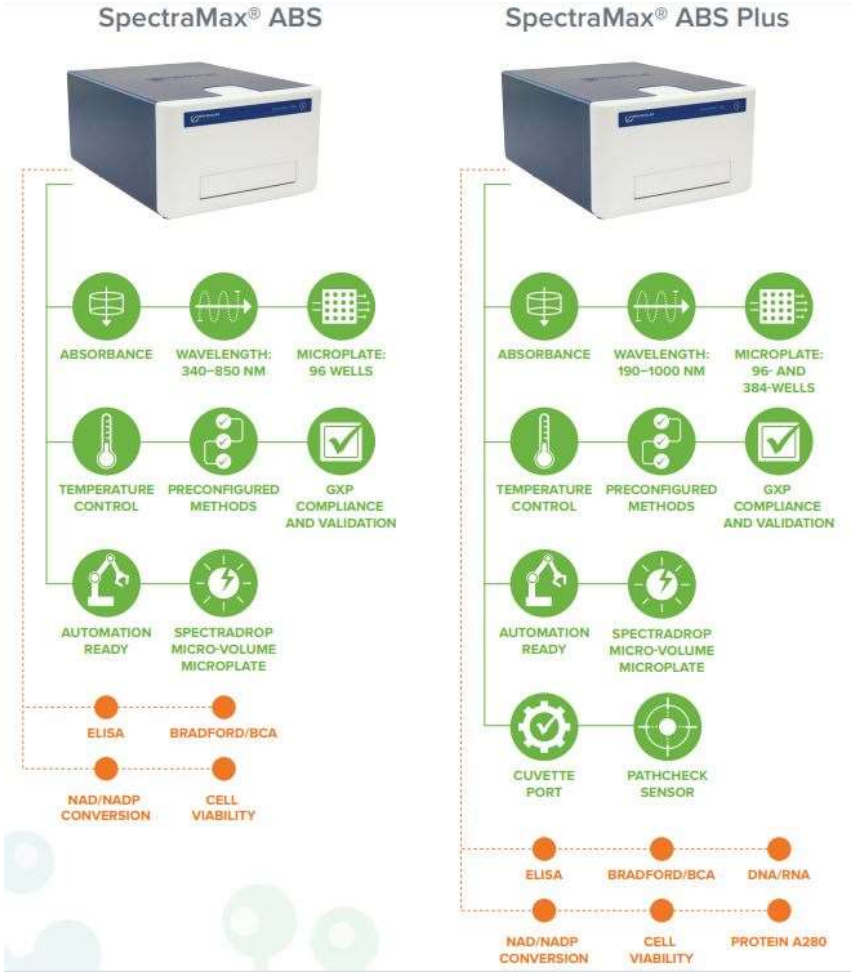
Available Upgrades
✓ Vis Absorbance
✓ UV/Vis Absorbance
✓ BRET / FRET

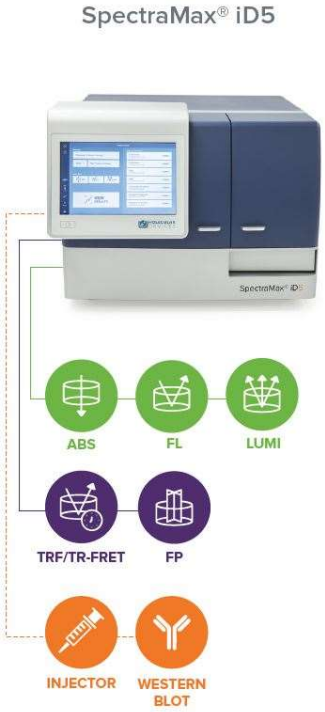
GloMax® Discover

6-, 12-, 24-, 48-, 96- and 384-well

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

Monochromator-Based Microplate Readers





Thank you for your attention!