Cell Reporter Systems

Discovering NanoLuc®

VWR Cell Culture Day

Sept 27th-28th, 2016

Promega Corporation

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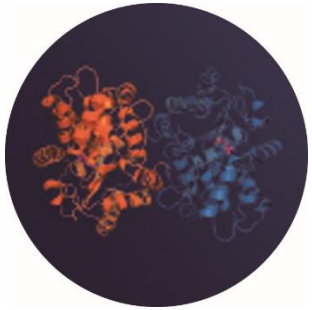
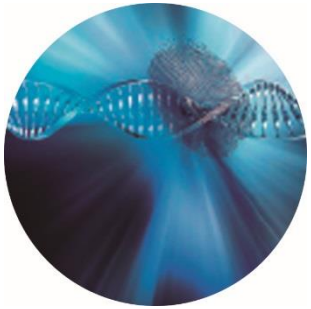
Our Products

Portfolio

Genomics
Nucleic acid purification, enzymes, RNase inhibitors, reverse transcription, PCR, real-time PCR, markers, cloning systems, transfection

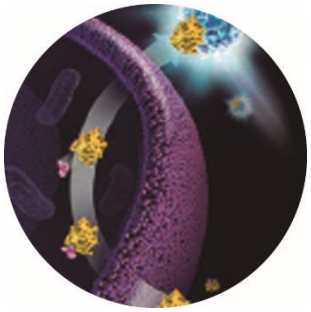
Protein Analysis
Protein expression and purification, live cell labeling and imaging, protein interaction assays, antibody labeling and purification, antibody fragmentation, Western Blotting, ELISA, reagents for mass spectrometry





Genetic Identity
Preprocessing and differential extraction, STR amplification and analysis, DNA isolation, human-specific DNA quantitation, automation for genetic identity

Portfolio

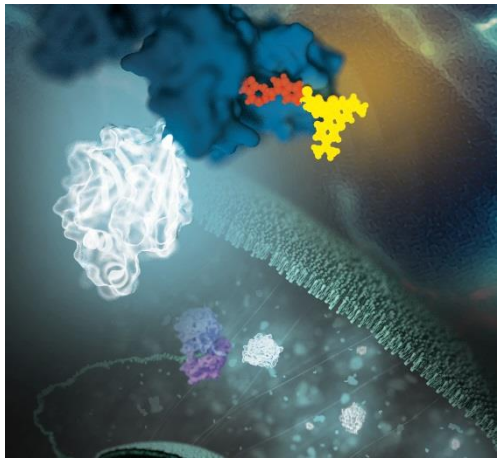


Cellular & Biochemical Assays
Viability, apoptosis, cytotoxicity, oxidative stress, cell signaling, kinases, epigenetics, real-time analysis, 3D-culture assays, cell metabolism, drug discovery, reporter gene assays

Instruments
GloMax® Discover and Explorer Multimode Systems for detection of luminescence, fluorescence, absorbance, BRET and FRET. Maxwell® for protein and nucleic acids purification. Quantus™ for DNA/RNA quantitation.

http://www.promega.es/products

Today we will focus in…



NanoLuc™ Luciferase Technology

- Bioluminescence
- What is NanoLuc™?
- NanoLuc™ as a reporter gene
- NanoLuc™ as a fusion partner

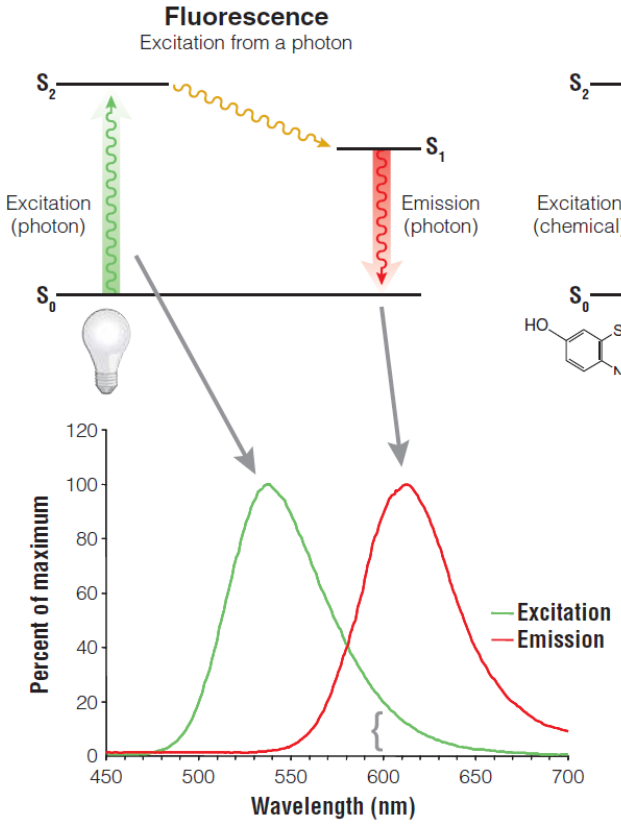
GloMax® Instruments

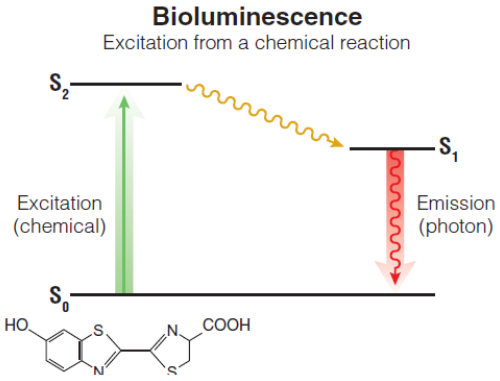
- Features & benefits
- Comparing devices
- Customize your assays
- Flexibility

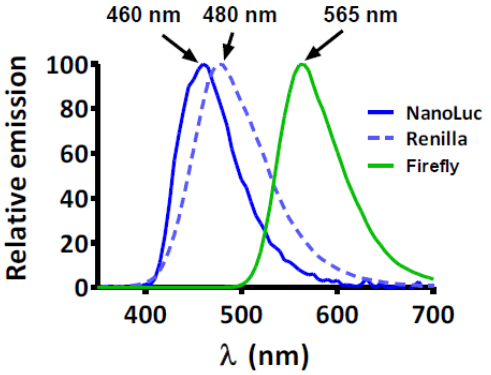
Bioluminescence in Life Sciences

Promega Corporation

The Physics of Bioluminescence





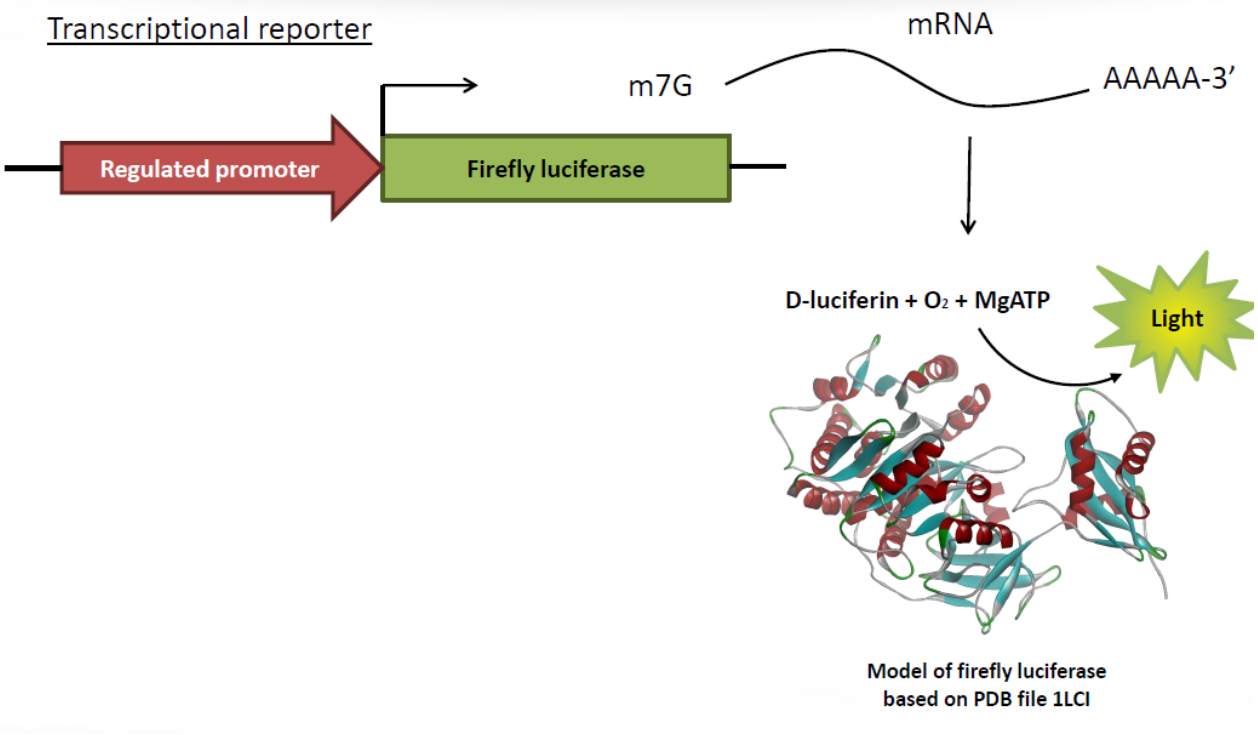


Luminescent assays avoid interferences that affect the sensitivity of fluorescent assays, including:

	Fluorescence	Bioluminescence
Photon Interference	+++	0
Cell fluorescence	+++	0
Media emission	++	0
Compound emission	+++	0
Filter quality	++	0

Bioluminescence in Life Science Research

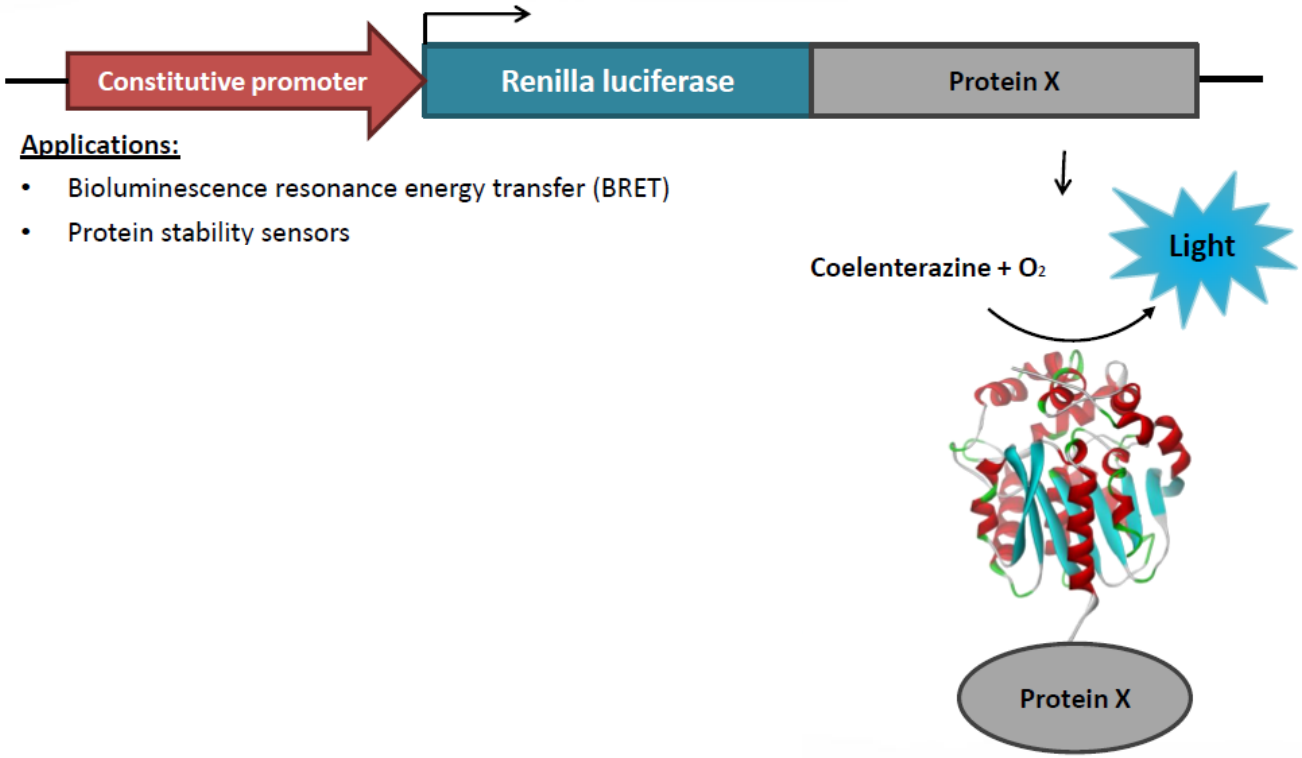
- Luciferase as reporter gene
- Luciferase as fusión partner



Model of firefly luciferase based on PDB file 1LCI

Bioluminescence in Life Science Research

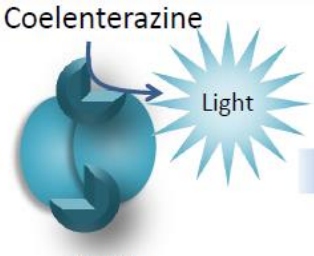
- Luciferase as reporter gene
- Luciferase as fusión partner



What is NanoLuc™?

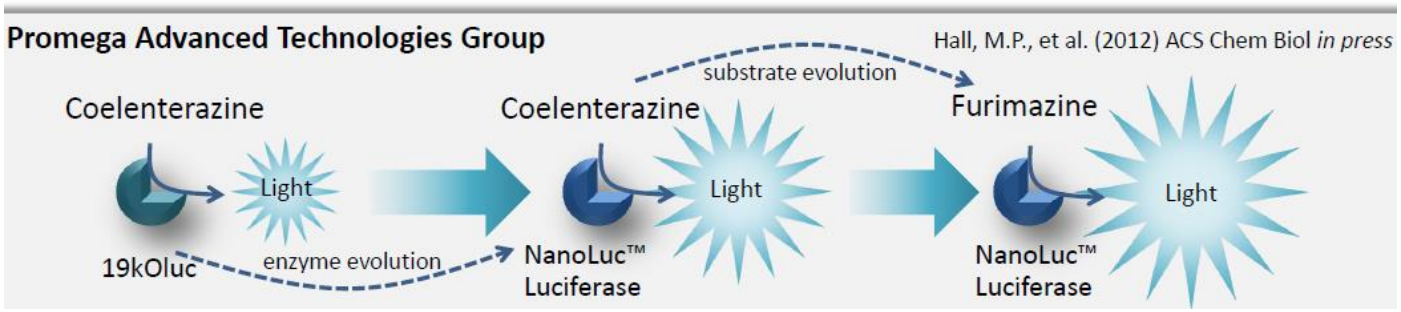
How was NanoLuc™ developed?

Oplophorus gracilirostris first cataloged in 1881



130kDa
Oplophorus luciferase
7X brighter than native
Renilla Luciferase
Shimomura, O., et al. (1978)

19kOluc
19kDa subunit is catalytic.
Light output & stability compromised.
Inouye, S., et al. (2000)



Sea pansy

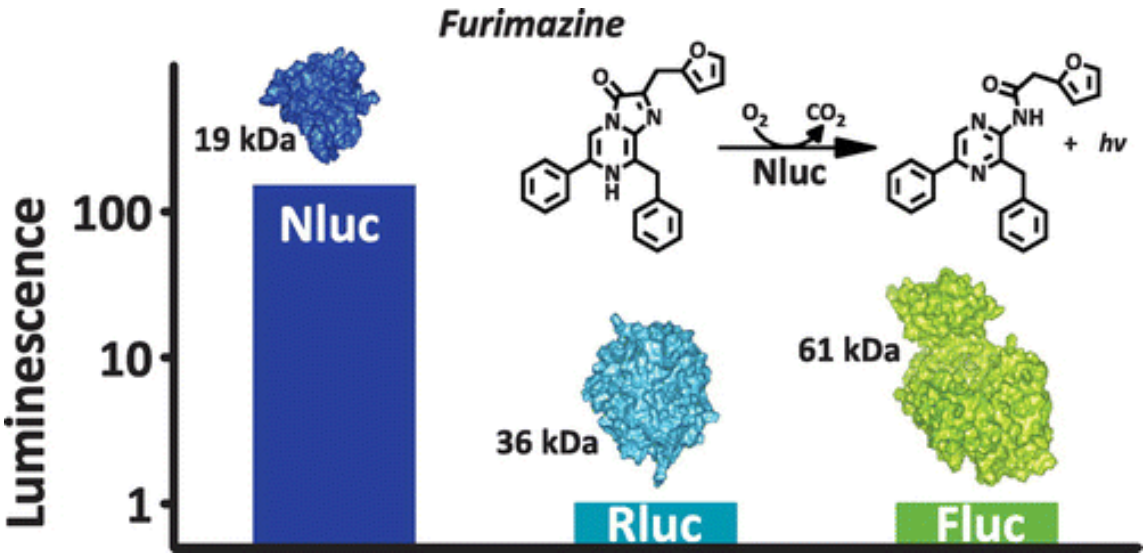
Scientific classification

Kingdom:	Animalia
Phylum:	Cnidaria
Class:	Anthozoa
Subclass:	Octocorallia
Order:	Pennatulacea
Family:	Renillidae
Genus:	**Renilla**
Species:	***R. reniformis***

Binomial name

Renilla reniformis

What is NanoLuc™?

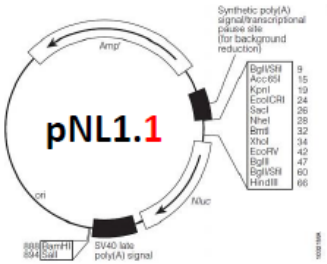


- ✓ Extremely Bright Output
- ✓ Incredibly Small Size
- ✓ Versatile Performance

A new, engineered luciferase enabling advanced reporter applications

NanoLuc™ as a reporter gene

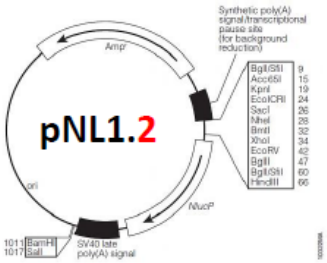
3 Varieties of NanoLuc™ Luciferase for you



Intracellular Formats

NanoLuc™ Luciferase

Nluc (513 bp)

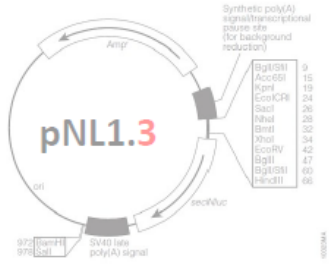


Protein destabilization domain

NanoLuc™ Luciferase | PEST

NlucP (636 bp)

Secretion Format



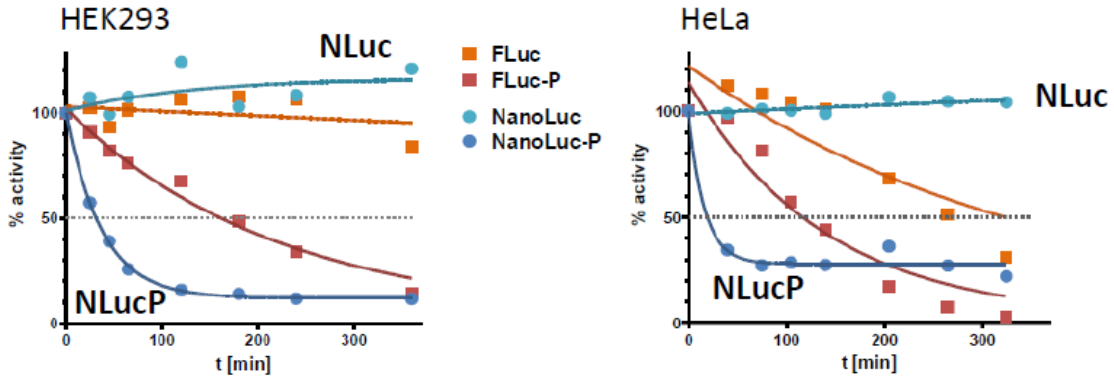
IL6 | NanoLuc™ Luciferase

secNluc (597 bp)

Secretion signal

Intracellular stability of NanoLuc™ and Firefly

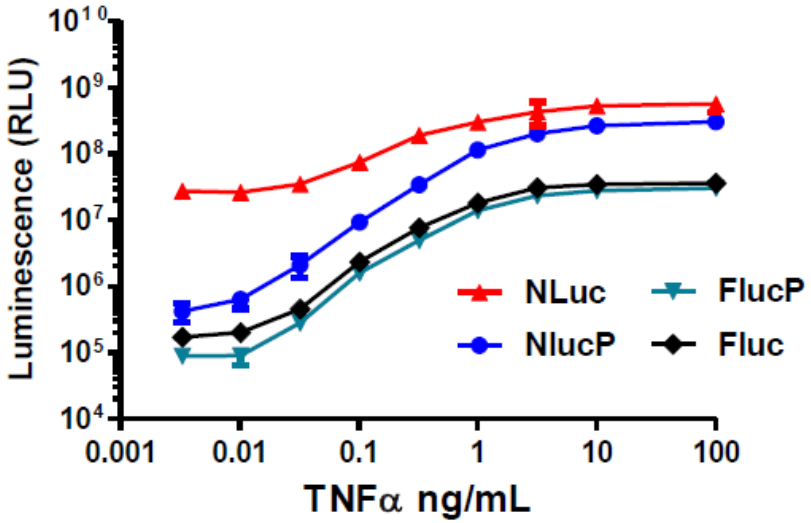
New protein synthesis blocked by addition of cycloheximide



Cell line	FLuc	FLucP	NLuc	NLucP
HEK-293	>6 h	2.0 ± 0.4 h	>6 h	18 ± 11 min
HeLa	3.8 ± 1.3 h	1.4 ± 0.2 h	>6 h	20 ± 6 min
U2OS (n=1)	>6 h	2.8 h	>6 h	36 min

Relative protein stability in cells: NlucP < FlucP < Fluc < Nluc

NlucP gives the greatest dynamic response



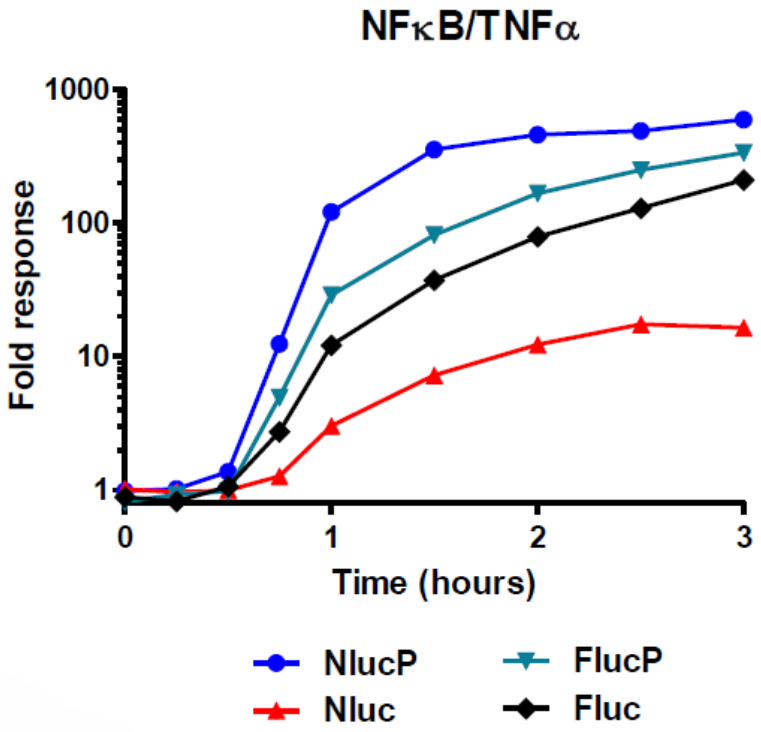
Experimental details: transient transfection of HEK293 cells with NF-κB inducible constructs. rhTNFα treatment for 5 hours.

Brightness

Nluc > NlucP > Fluc > FlucP

→Very similar pharmacology/EC50s

NlucP responds earliest to stimuli

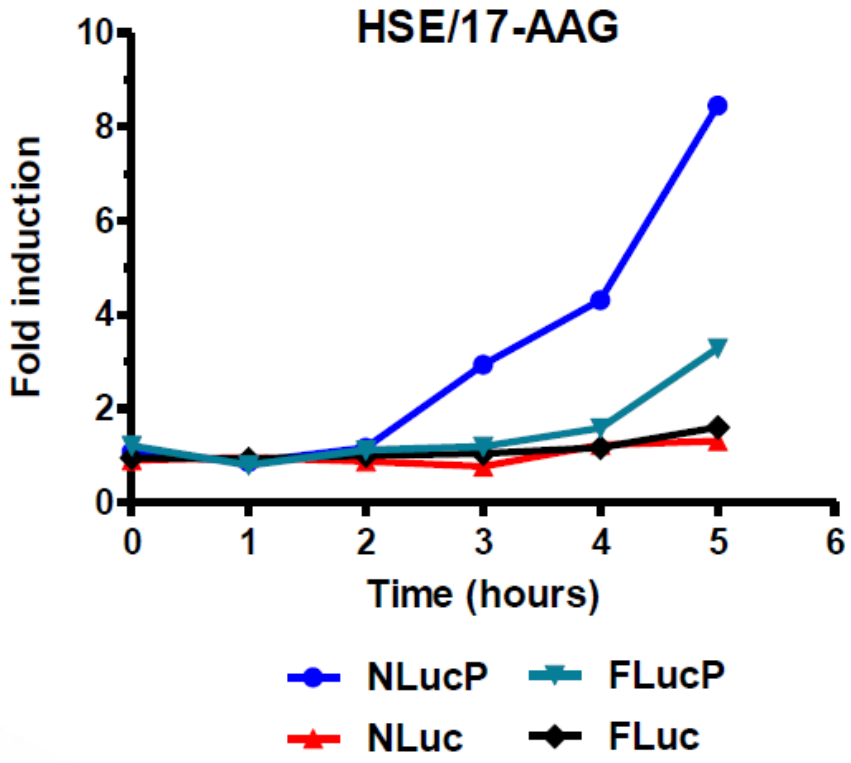


Relative Response

NlucP > FlucP > Fluc > Nluc

Experimental details: transient transfection of HEK293 cells with NFκB inducible constructs; addition of 100 ng/ml rhTNFα at time zero.

NLucP allows study of weakly induced responses



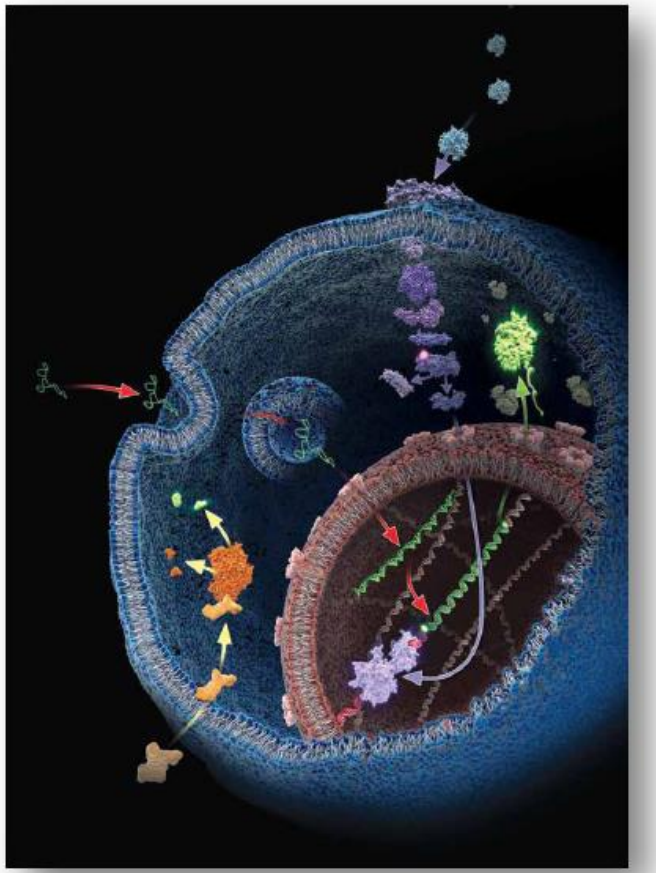
Relative Response

NlucP > FlucP > Fluc,Nluc

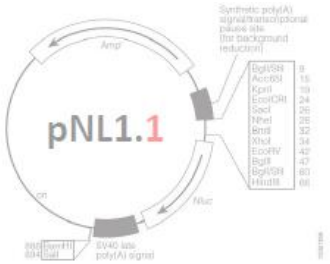
Experimental details: transient transfection of Hela cells w/ Hsf1 inducible constructs; addition of 500 nM 17-AAG at time zero.

NanoLuc Luciferase as an intracelular reporter

- ✓ NlucP for a faster response
- ✓ NlucP for greatest dynamic range
- ✓ NlucP for measuring weak responses
- ✓ Nluc where maximum brightness is needed.



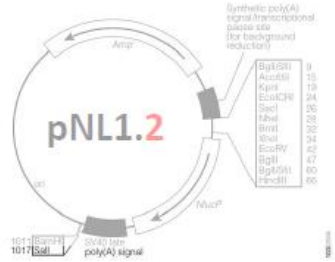
3 Varieties of NanoLuc™ Luciferase for you



Intracellular Formats

NanoLuc™ Luciferase

Nluc (513 bp)

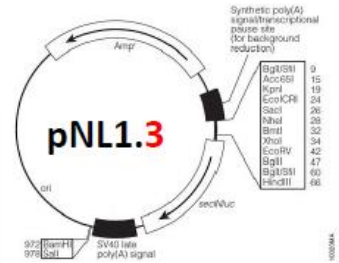


Protein destabilization domain

NanoLuc™ Luciferase | *PEST*

NlucP (636 bp)

Secretion Format

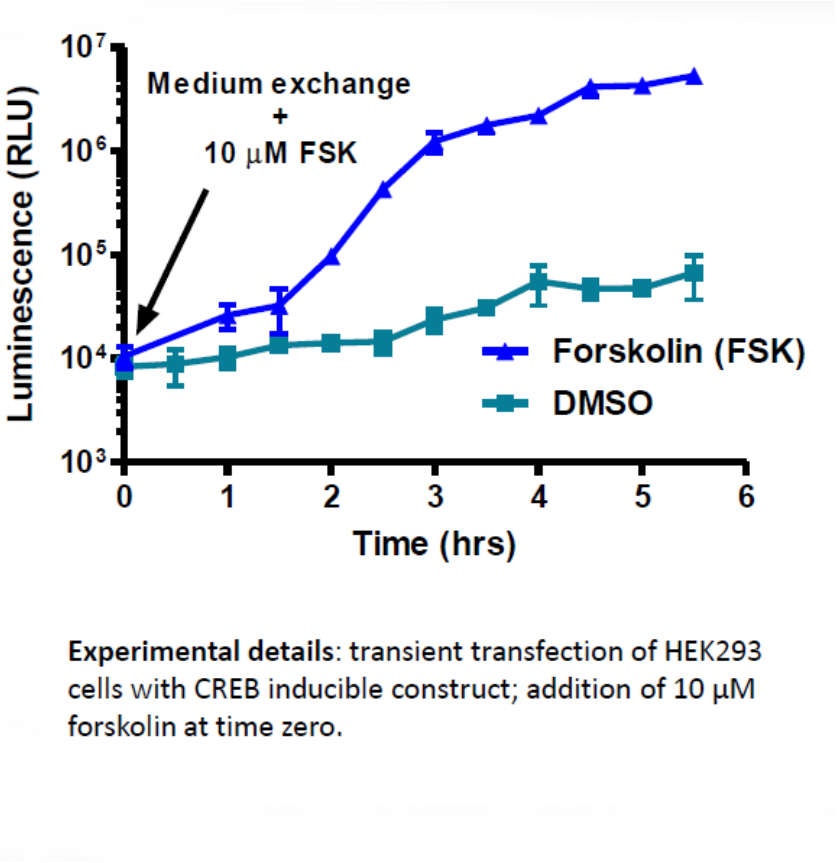


IL6 | *NanoLuc™ Luciferase*

secNluc (597 bp)

Secretion signal

Secretion based format using secNluc



Experimental details: transient transfection of HEK293 cells with CREB inducible construct; addition of 10 µM forskolin at time zero.

Should I switch from Firefly to NanoLuc™ Luciferase?

Does it allow you to do your work?
Do you plan to do work in vivo?

Firefly is a great reporter

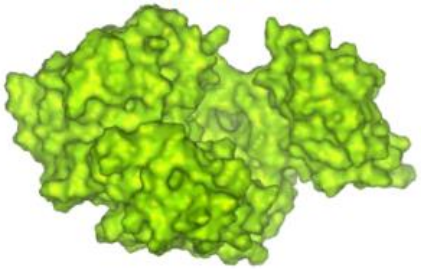
- ✓ Excellent signal:background
- ✓ Excellent dynamic range

We just released new response element signaling pathway detection pGL4 vectors:

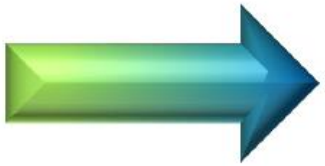
ARE	HSE	ISRE	STAT5	SRE
p53	HRE	SIE	NFAT	SRF
ATF6	XRE	SBE	CRE	
MRE	AP1	TCF-LEF	NF-κB	

Should I switch from Firefly to NanoLuc™ Luciferase?

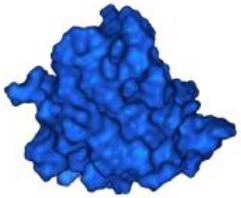
Firefly (Fluc)



Yes, if ...



NanoLuc™ (Nluc)



- ✓ **Transfection efficiency limits you to easy-to-transfect cell lines**
- ✓ **Signals are too weak to move to 96-well plates**
- ✓ **FLuc is just too big**

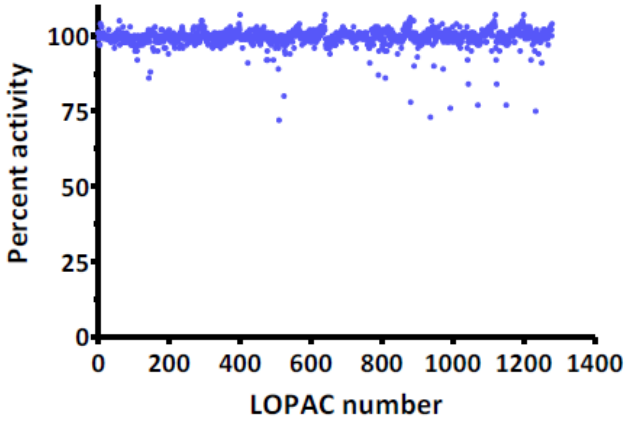
- The increased brightness could allow a subtle signal become a reliable signal.
- The small size could allow gene replacement with minimal impact, especially in viral constructs

Primary cells with **poor transfection** or expression from **endogenous** promoters

Reduced false hit rate in HTS

LOPAC library (Sigma)

- 1280 compounds
- Small organic ligands w/ well documented pharmacological activities
- Used to screen for non-specific luciferase activity modulators

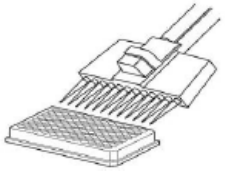


		Level of inhibition			
		≥ 10%	≥ 20%	≥ 30%	≥ 50%
% of library compounds	NanoLuc	1.2%	0.5%	-	-
	Firefly	1.9%	0.7%	0.5%	0.3%

Experimental details: LOPAC library members at 10 µM final concentration; incubation with purified NanoLuc or firefly luciferase for 2 min.; Fluc detection using Bright-Glo.

Nano-Glo™ Luciferase Assay Reagent

Standard format

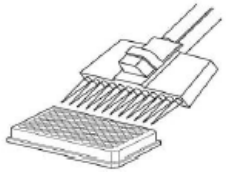


Add Nano-Glo Luciferase Assay Reagent

Measure RLUs after 3'

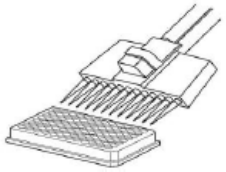
1) Cells lysed
2) Intracellular Nluc released

Secretion format



Transfer aliquot of medium

New plate



Add Nano-Glo Luciferase Assay Reagent

Measure RLUs after 3'

1) Nluc secreted from cells
2) Sample culture medium (no cell lysis)

Nano-Glo® Dual-Luciferase® Reporter Assay (NanoDual)

The most sensitive, most powerful dual-reporter assay available.

> **Improved Performance:** Better signal quenching for two fully dynamic reporter options and more consistent data.

> **Greater Sensitivity:** Better detection of small changes, less interference with natural biology, and the best signal:background available in a dual-reporter assay.

> **Less Control DNA; More Reliable Data:** A brighter control reporter means you use less control DNA, and may minimize assay artifacts.

> **More Choice in Assay Design:** Use firefly, NanoLuc or both as primary reporters; use with injectors or in add-mix-read format.

NanoLuc™ Firefly

Multiple Assay Configuration Options

NanoLuc® Luciferase Primary Reporter

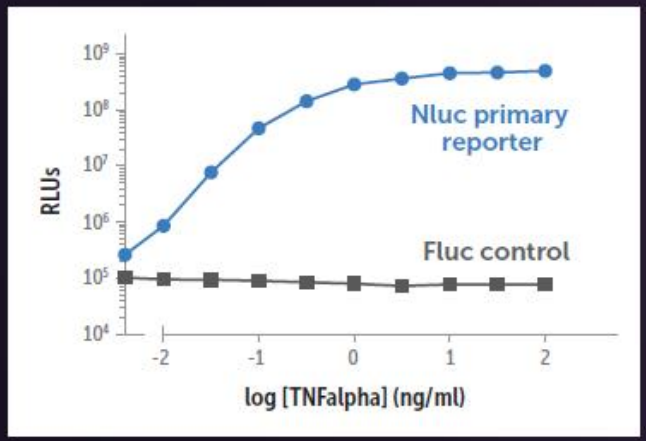
The brightest, most responsive format.

Best for:

Low cell numbers, plate scale-up or challenging cell lines.

Reporter Constructs:

- NlucP Experimental Reporter »
- Fluc Control »



NanoLuc® Luciferase Primary Reporter. Data shows luminescence values for HEK293 cells transfected with NFkB-NanoLuc® PEST vector and TK-Fluc control at 10:1 ratio. Cells were treated with TNFα and reporter activity measured using the NanoDLR™ assay 4 hours post-treatment.

Multiple Assay Configuration Options

Firefly Luciferase Primary Reporter

Use existing firefly luciferase constructs, and add NanoLuc® luciferase for a more robust control.

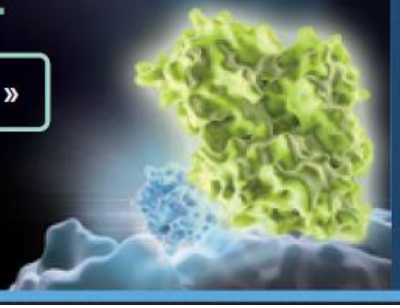
Best for:

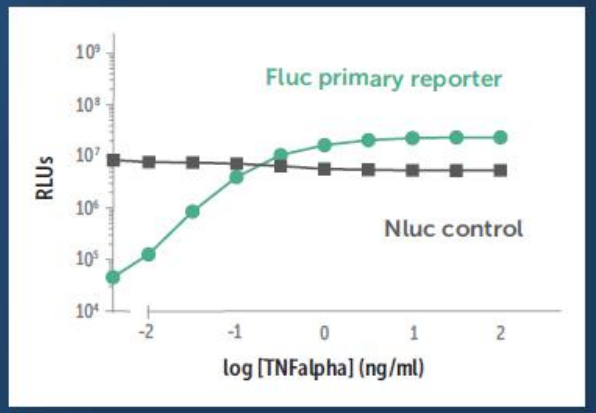
Standard reporter assay formats. The NanoLuc® luciferase control reporter is up to 1000x brighter than *Renilla* luciferase, so you can use less control DNA.

Reporter Constructs:

FlucP Experimental Reporter »

Nluc Control »





Firefly Luciferase Primary Reporter. Data shows luminescence values for HEK293 cells transfected with NFkB response element Fluc-PEST vector and a TK-NanoLuc® control vector at 10:1 ratio. Cells were treated with TNFα and reporter activity measured using the NanoDLR™ assay 4 hours post-treatment.

Multiple Assay Configuration Options

Two Primary Reporters: Two Biological Responses

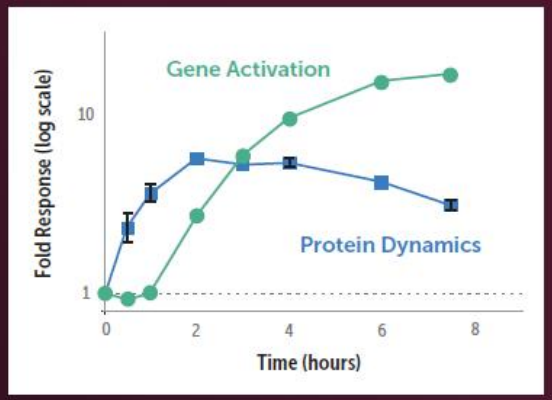
Measure two different biological responses simultaneously.

Best for:

Measuring two pathways at once for maximum data; multiplexing protein stability and genetic reporter assays.[1]

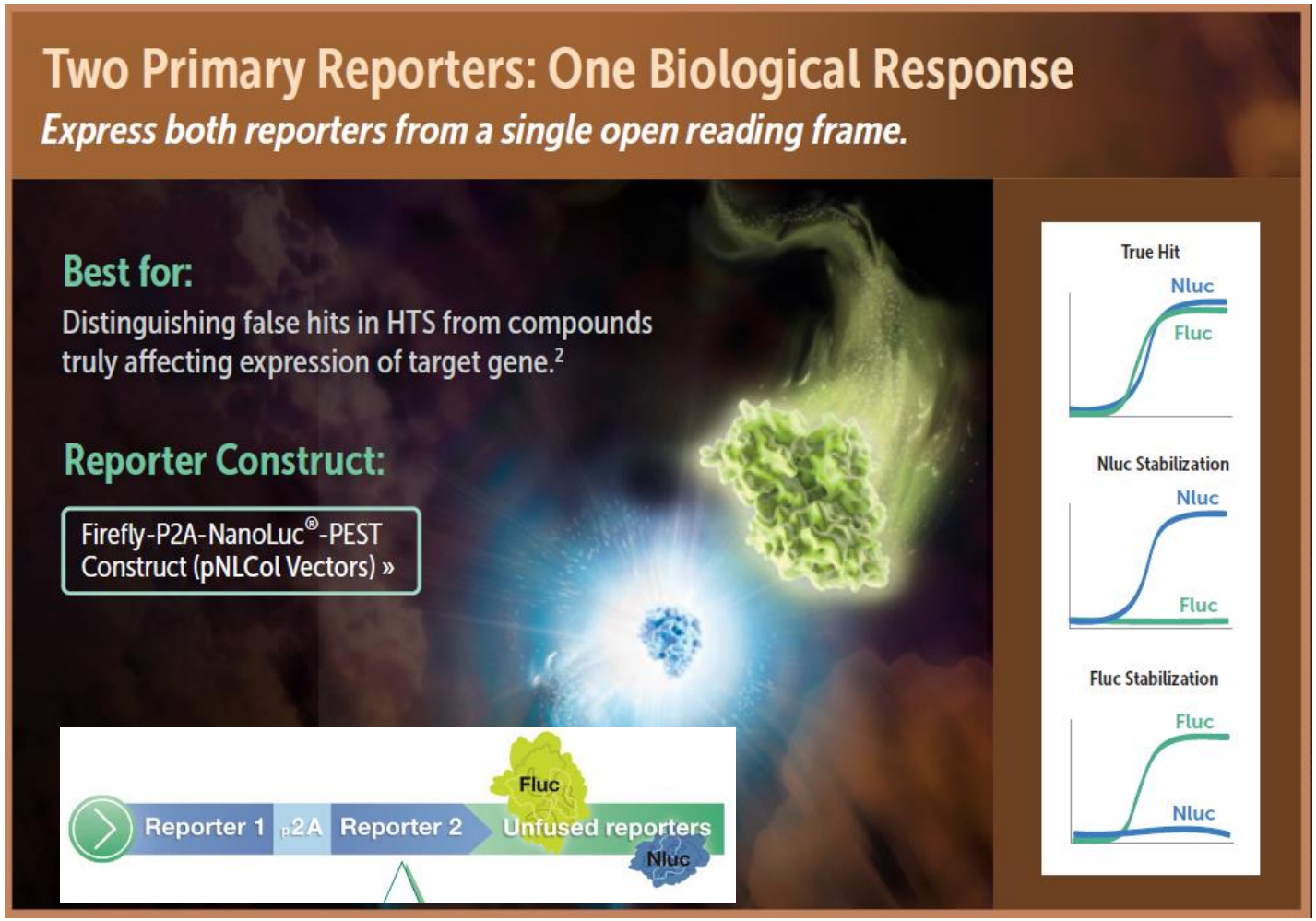
Reporter Constructs:

- Nluc Experimental Reporter »
- FlucP Experimental Reporter »
- Nluc Protein Stability Reporter »



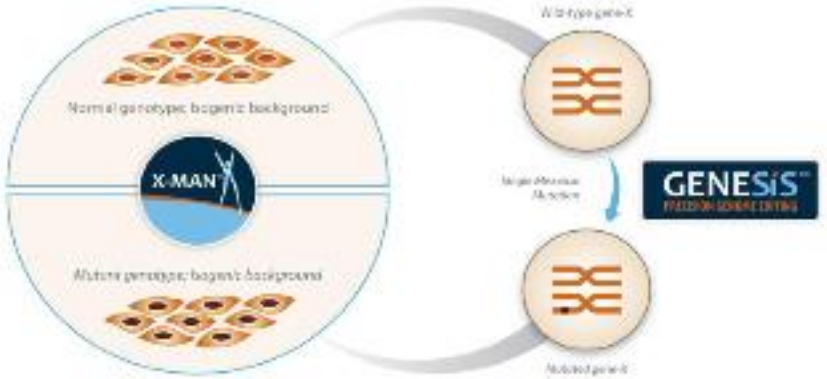
Measure Protein Dynamics and Gene Activation in the Same Sample. HEK293 cells were transiently transfected with pNLF1-HIF1A[CMV/neo] fusion construct, diluted 1:1000 into the Hypoxia Response Element Vector, pGL4.42[luc2P/NRE/Hygro]. After 18 hours, cells were stimulated with varying doses of 1,10-phenanthroline and assayed for expression of the firefly luciferase transcriptional reporter and the HIF1a-NanoLuc® fusion protein using the Nano-Glo® Dual-Luciferase® Reporter (NanoDLR™) Assay at the indicated time points.

Multiple Assay Configuration Options



NanoLuc® as robust reporter for endogenous gene regulation

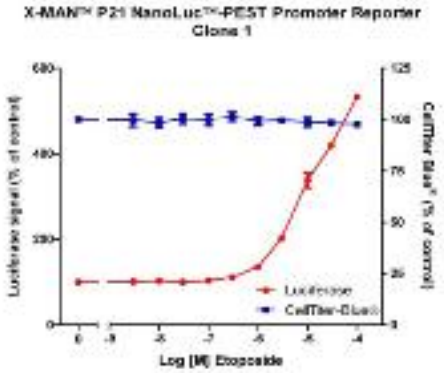
Horizon Discovery Gene Editing Technology



- Precisely introduce genetic changes to endogenous genes
- Enables real time monitoring of gene or protein regulation *in vivo* NanoLuc technologies

Monitoring Endogenous P21 Transcriptional Regulation Using NanoLuc Reporter

P21-NlucP HCT116 Cell Line

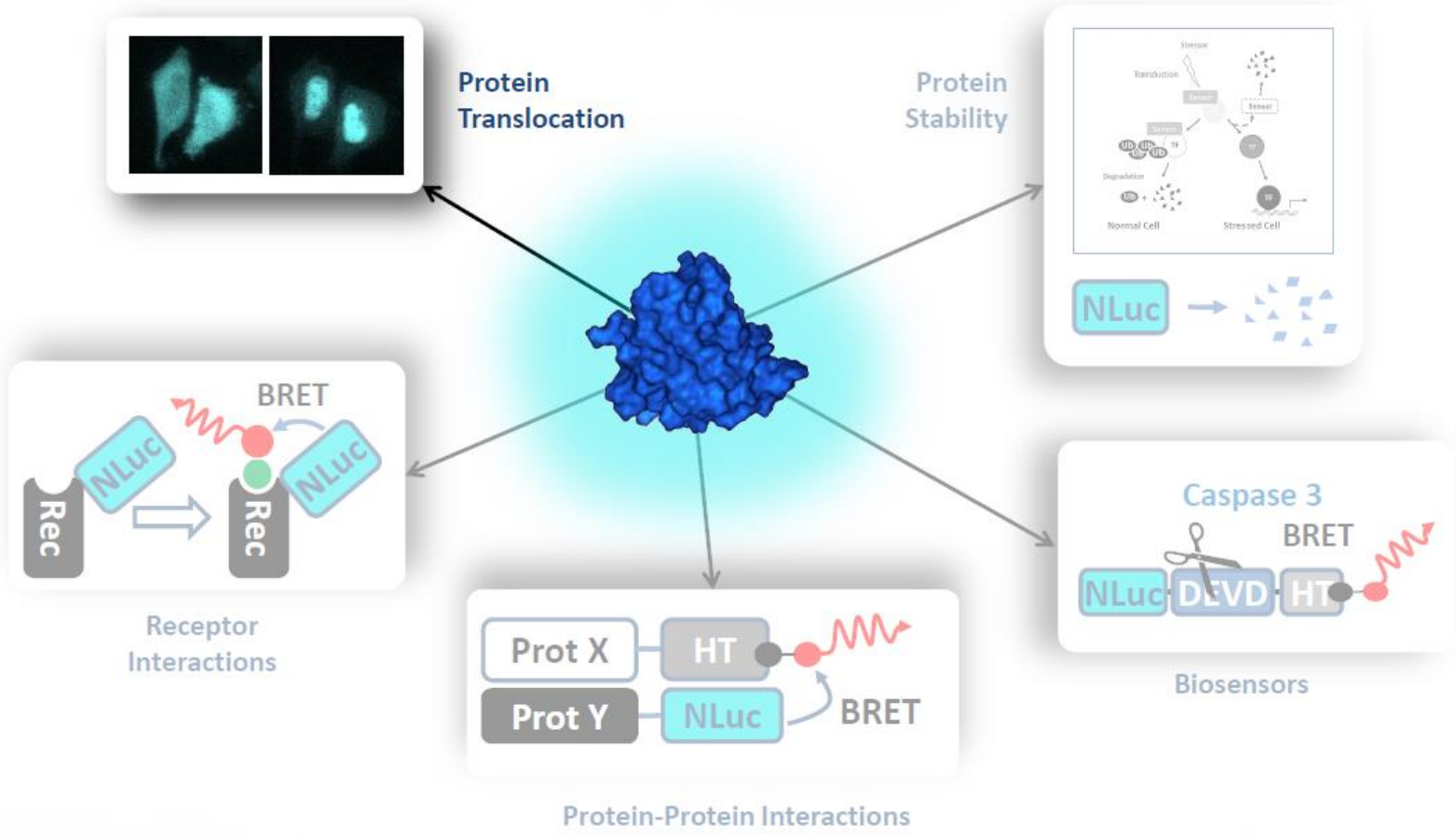


Specific activation of p21 by etoposide can be detected by the NanoLuc reporter. A multiplexed cell viability assay was also performed. N=3.0

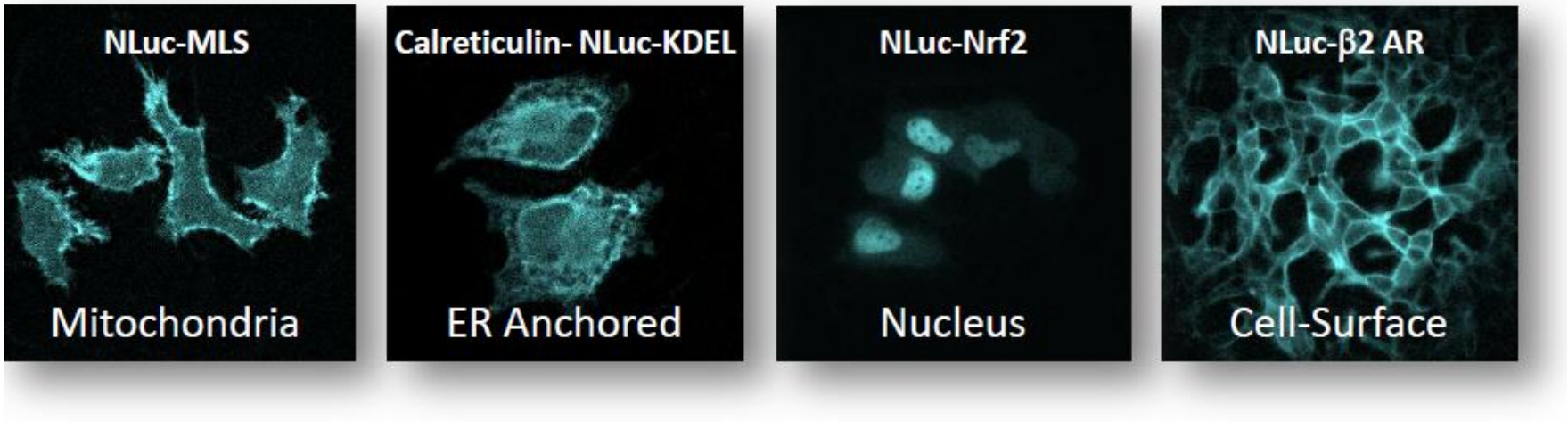
NanoLuc™ Luciferase as a fusion partner

Promega Corporation

Proof of concept experiments



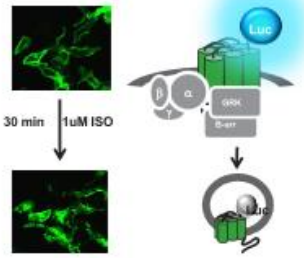
NanoLuc™ Fusions can go anywhere…



NanoLuc™ Luciferase fusions could be a useful tool to investigate cell biology

Monitor endocytosis using NanoLuc™

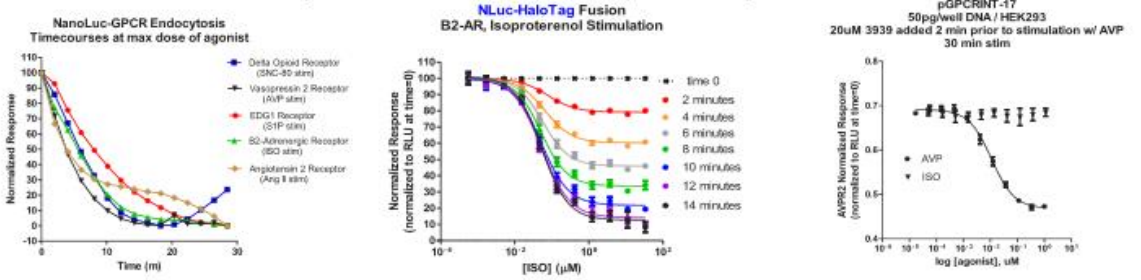
Schematic Diagram of Receptor Endocytosis



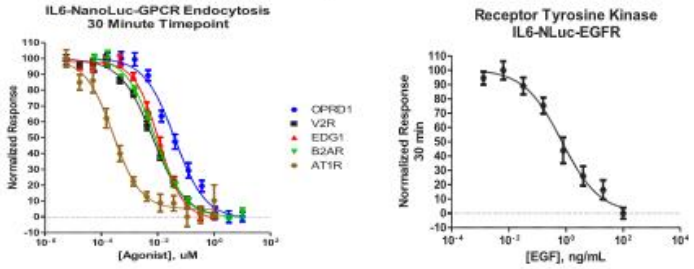
Receptor Endocytosis Assay:

- Live Cell, kinetic, plate-based assay
- Bright, small tag, allows low expression for proper pharmacology
- Loss of signal after receptor endocytosis
- No engineering of receptor c-term or beta arrestin
- Compatible with transiently transfected cells

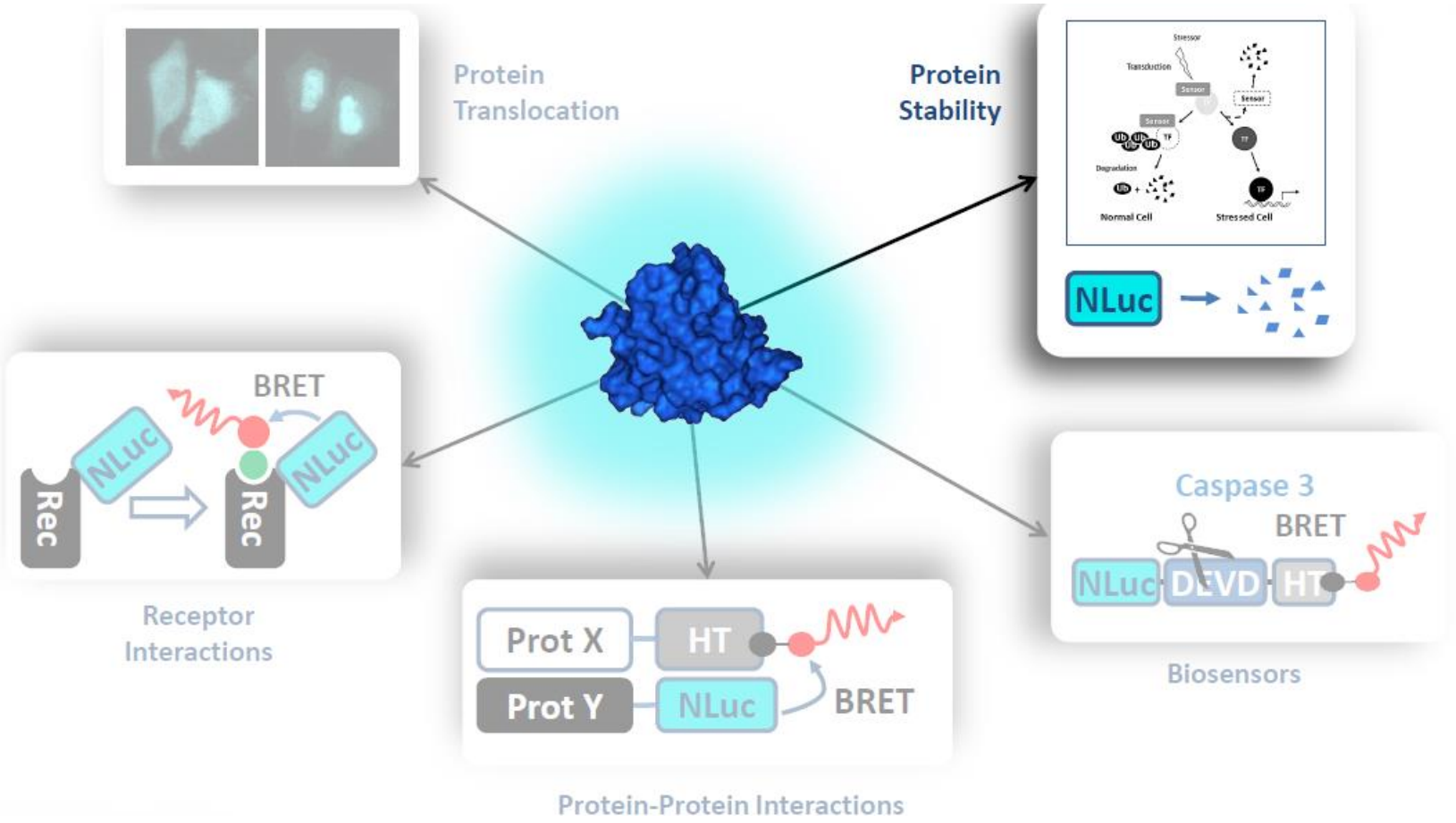
Dynamic Measurement of Receptor Endocytosis



Nluc Receptor Endocytosis Assay Can Be Expanded to Both GPCRs and RTKs

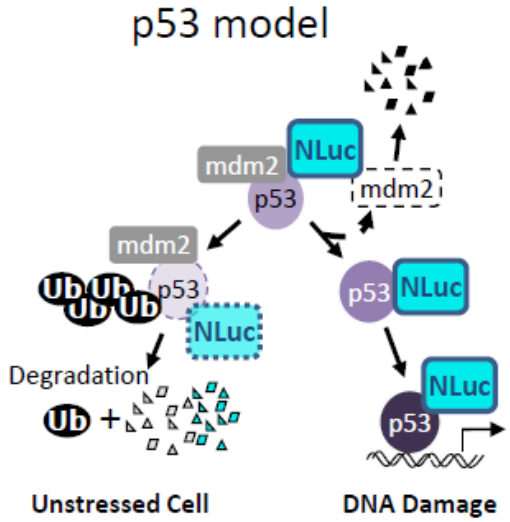


Nanoluc™ Luciferase as a fusión partner

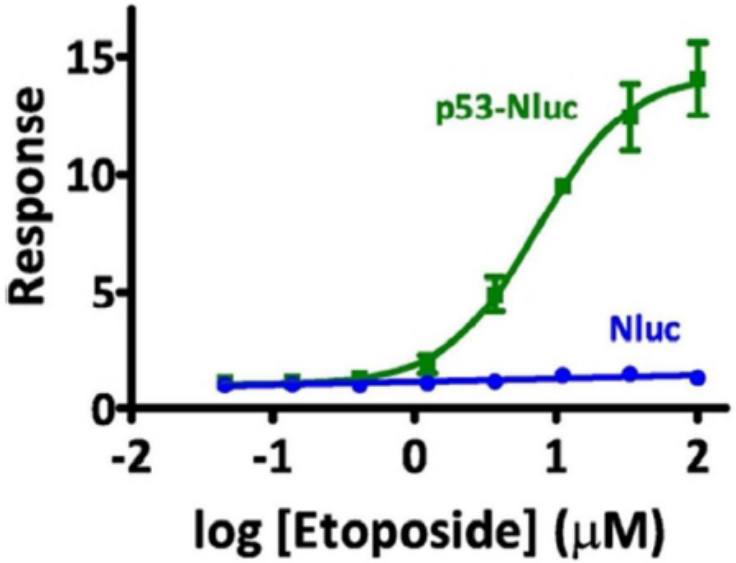


Monitoring Protein Stability with NanoLuc™ Luciferase

Can NanoLuc™ Luciferase be added to a protein as a probe for protein stability?

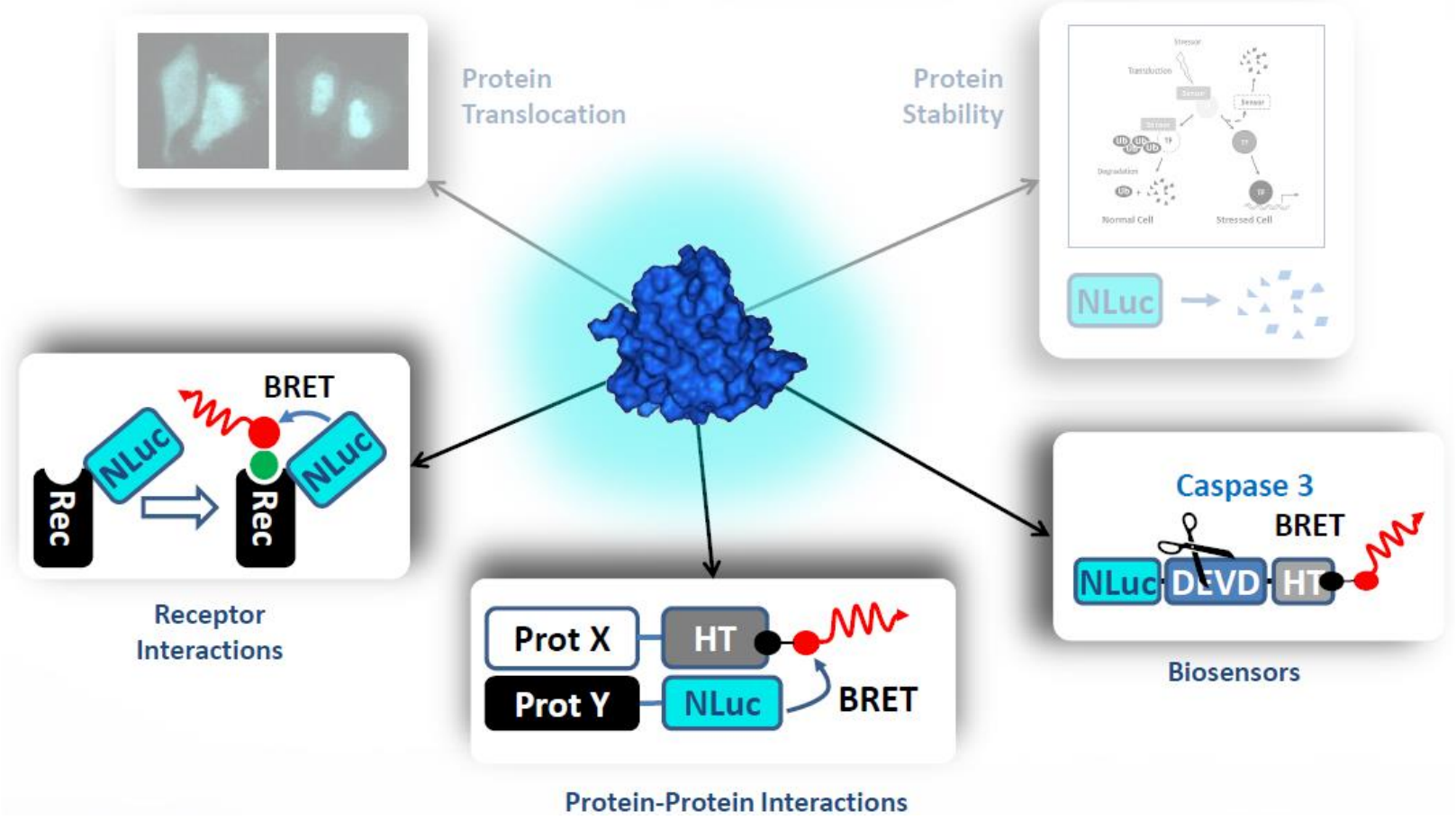


Endogenous levels

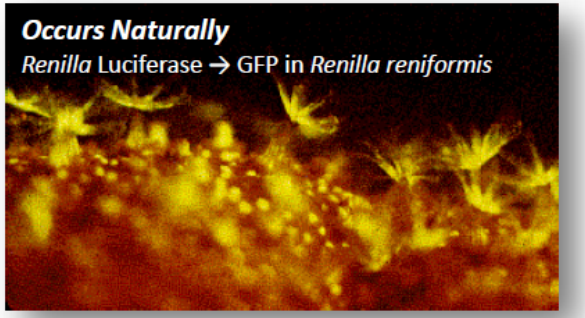


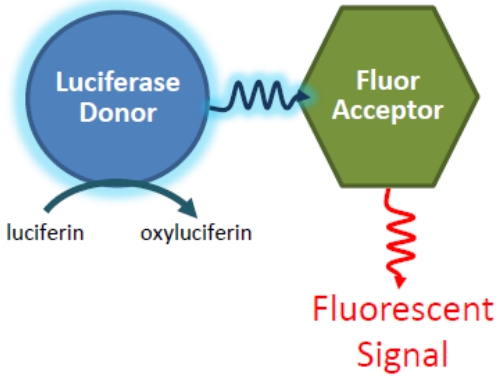
The fusion can be used as a probe of stability

Nanoluc™ Luciferase as a fusión partner



Bioluminescence Resonance Energy Transfer (BRET)





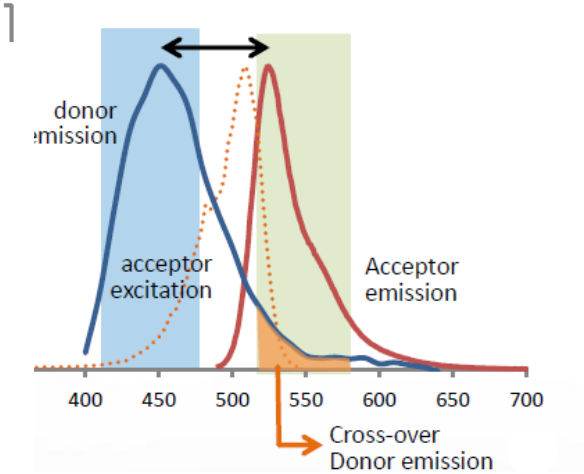
Important characteristics for BRET applications in research:

RLuc → GFP

Donor brightness is a key limiter to current BRET technologies.

BRET-beneficial aspects of NanoLuc Luciferase:

~100-fold brighter than Rluc
- ✓ need less spectral overlap with fluor
- ✓ gain greater spectral separation

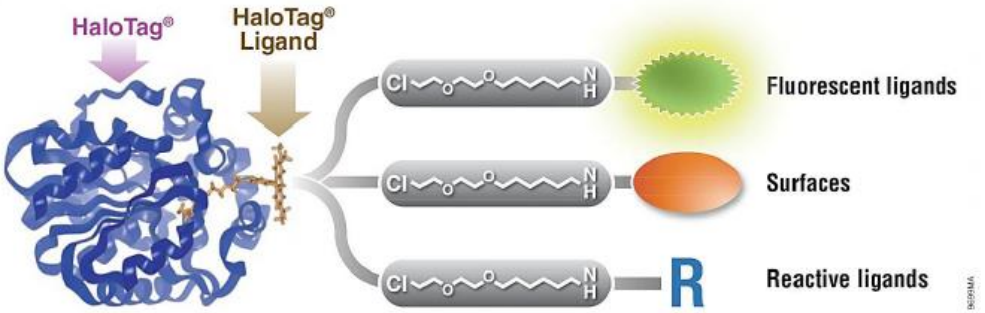


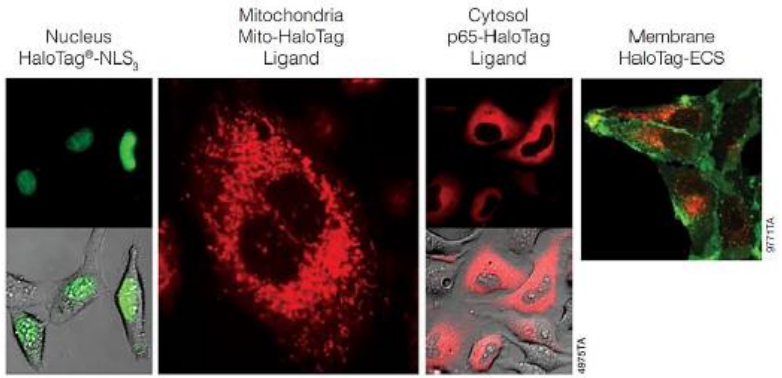
We have a potential acceptor fusion protein:

HaloTag® Fusion Protein

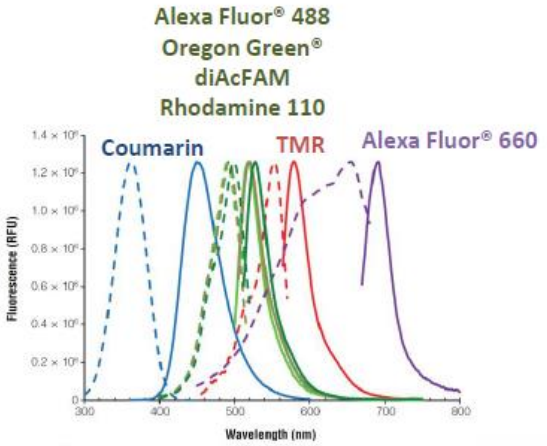
34.1kDa protein engineered from halophilic bacterial hydrolase.

- Engineered to lock into enzyme: substrate intermediate for **covalent attachment** .
- No homolog in mammalian cells.





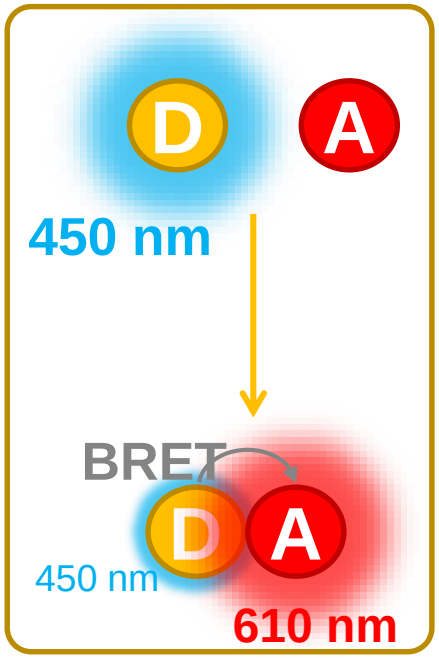
Goes anywhere in the cell

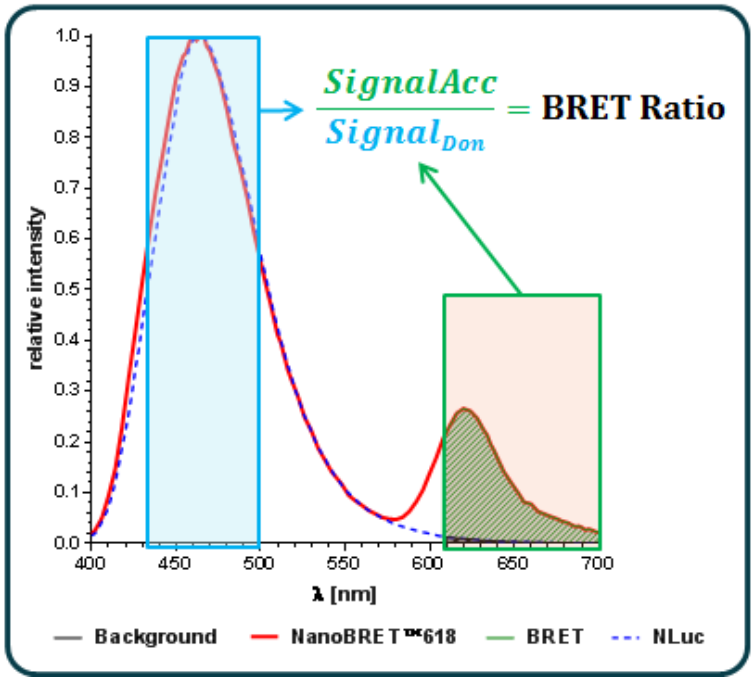


Variety of fluors ready-to-use

Monitor and Screen Intracellular Protein-Protein Interactions Using NanoBRET™ and GloMax® Discover

- BRET provides real time measurement *in living cells*
- Superior luminescent Donor signal from NanoLuc™
- Flexible choice of Donor/Acceptor Separation
- Low Donor/Acceptor ratios provides best dynamic range





NanoBRET™ 618

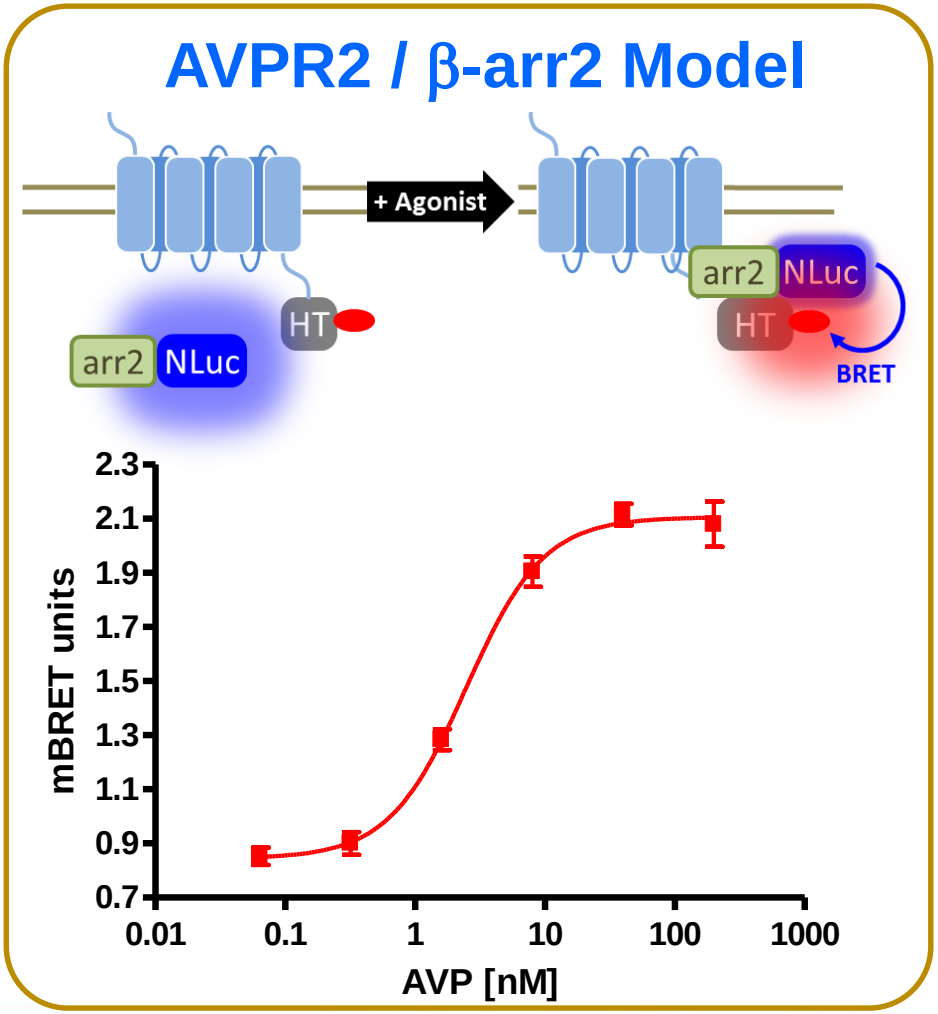
Express Donor and Acceptor protein fusions

Label Cells with HaloTag (Acceptor fusion)

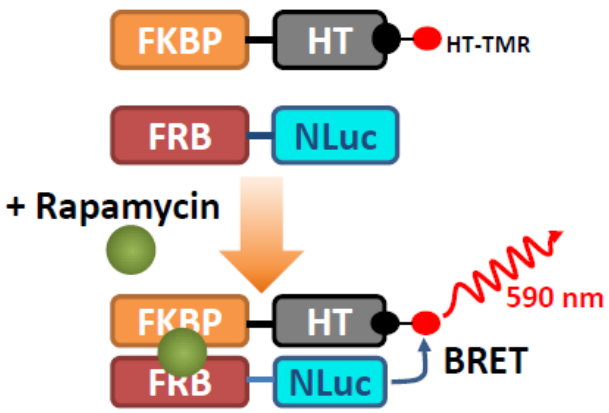
Induce interaction

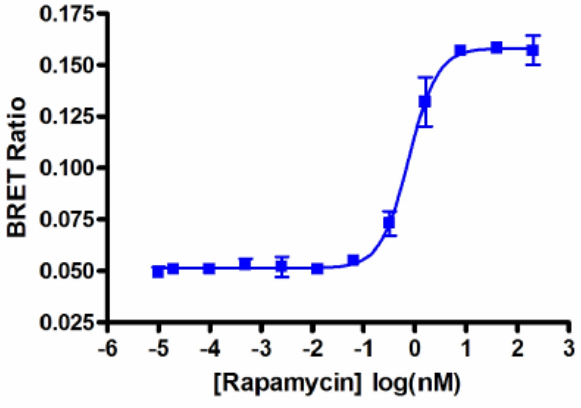
GloMax® Discover Detection System

Monitor and Screen Intracellular Protein-Protein Interactions Using NanoBRET™ and GloMax® Discover



Nluc:HT Pair can be used for protein-protein BRET

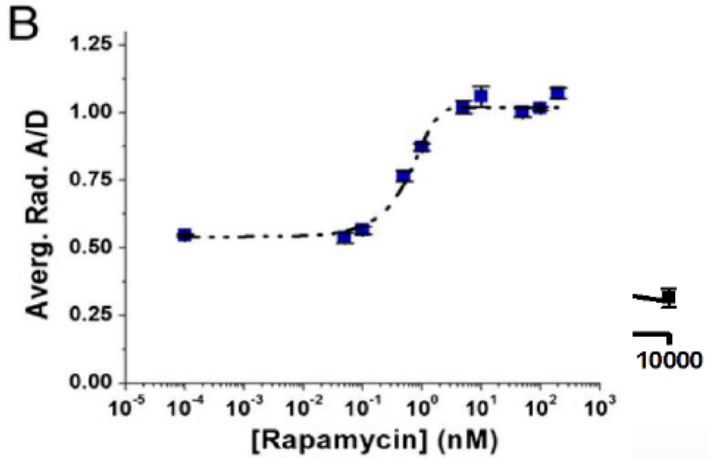




Same model system used with BRET 6 System

RLuc8.6 → TurboFP

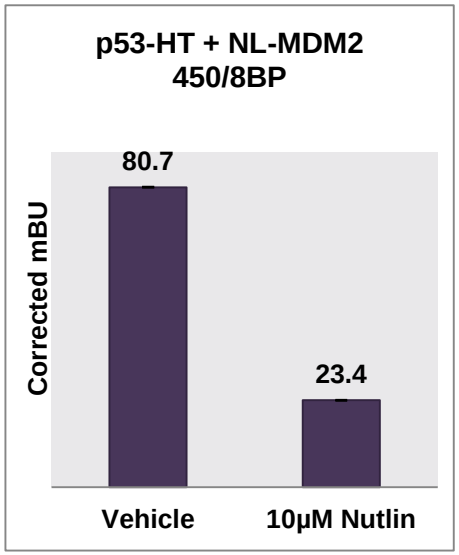
Dragulescu-Andrasi, A., et al (2011) *PNAS* **108**, 12060-5.



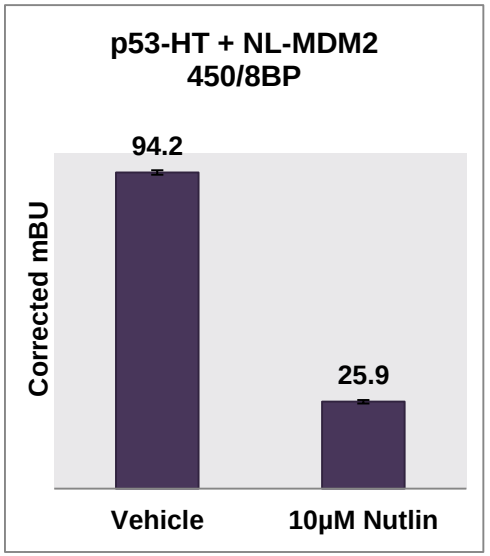
Monitor and Screen Intracellular Protein-Protein Interactions Using NanoBRET™ and GloMax® Discover

- GloMax® Discover shows excellent BRET response in in both 96 and 384-well plate formats
- GMD is the instrument of choice for NanoBRET, providing the best value for customers

GloMax® Discover



96-well format



384-well format

Watch the webinar

GloMax® instruments

Promega Corporation

Getting the Most From Your Plate-Based Assays

- Scientific discoveries involve not only finding the right assay, but also the right instrument.
- *Assay sensitivity*, *dynamic range*, *sample-to-sample cross-talk*, and *ease-of-use* are critical considerations when choosing an assay and instrument.
- Instruments with poor optical performance, hinder assay performance and experimental design
- Promega develops and QC tests many of it's luminescence assays using GloMax® instruments, because of their:
  - Superior luminescence performance
  - World-wide acceptance and publications in peer-reviewed research journals

GloMax® Systems

GloMax® Systems: Simplify Your Research

It's Simple to Produce your Data

- Intuitive Touchscreen Display
- Pre-loaded Promega Protocols
- Automatic Gain Adjustment

Integrated with Promega Assays

- Optimized and Pre-loaded Promega Assays
- Spend less time optimizing your experiment

Superior Performance

- Broader dynamic range to measure extreme ranges
- Better sensitivity for low-level samples
- Lower well-to-well cross talk for confidence

Flexible

- Modular design so you choose
- the capability you need
- Automation-friendly to support
- your workflow
- 6 to 384-well plate formats

GloMax® Systems

GloMax® Navigator System

- Dedicated 96-well Luminometer
- Industry-leading luminescence performance
- Integrated Tablet PC and software
- Part of the *GloMax Systems* family
- State of the art motion control, electronics, PMT
- USB port connections
- Affordable price-point
- part 11 software capability
- IQ/OQ Service products are available

GloMax® Navigator

GloMax® Systems: Simplify Your Research

GloMax® Explorer

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

UPGRADE WHEN YOU NEED MORE

GloMax® Discover

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

*Note: upgrades are via instrument trade-in

GloMax® Systems: Simplify Your Research

Model	Lum	Fluor	Vis Abs	UV-Vis Abs	BRET / FRET
GloMax Navigator GM2000 / GM2010	✓				
GloMax Explorer GM3510	✓	✓	*Upgrade*	*Upgrade*	*Upgrade*
GloMax Explorer GM3500	✓	✓	✓	*Upgrade*	*Upgrade*
GloMax Discover GM3000	✓	✓		✓	✓

*Note: upgrades are via instrument trade-in

Integrated with Promega Assays

The Perfect Partner for Promega Assays

Preloaded Promega protocols or customize your own

Cell Signaling & Metabolism Assays: Including:

- ADP-Glo™
- Kinase-Glo®
- P450-Glo™
- cAMP-Glo™

Cell Health Assays: Including:

- CellTiter-Glo®
- CellTox™ Green
- Caspase-Glo®
- BacTiter-Glo®
- RealTime-Glo®

…plus many, many more, and multiplex

Luciferase Reporter Assays: Including:

- Nano-Glo®
- ONE-Glo™
- Dual-Glo® & DLR
- Nano-Glo® DLR
- Bright-Glo™
- ADCC Reporter Bioassay

BRET and FRET Assays:

Including:

- NanoBRET™
- Renilla/YFP
- Commercial and Homebrew assays

Extensive List of Applications on Promega.com

Application Notes: GloMax® Discover System

GloMax® Discover System

Bioassays

Measuring the ADCC Reporter Bioassay Complete Kit (WIL2-S) Signal on the GloMax® Discover System

Cell Health and Metabolism

Measuring the Output of the CytoTox-Fluor™ Cytotoxicity Assay on the GloMax® Discover System

Measuring the ONE-Glo + Tox Luciferase Reporter and Cell Viability Assay on the GloMax® Discover System

Measuring Cell Viability Using the CellTiter-Glo® Cell Viability Assay and GloMax® Discover System

Measuring P450-Glo™ Assays on the GloMax® Discover System

Measuring Bacterial Cell Viability Using the BacTiter-Glo™ Assay and GloMax® Discover System

Measuring Fluorescence Using the Apo-ONE® Homogeneous Caspase-3/7 Assay with the GloMax® Discover System

Measuring Fluorescence Using the CellTiter-Blue® Cell Viability Assay with the GloMax® Discover System

Measuring Fluorescence Using the ApoTox-Glo™

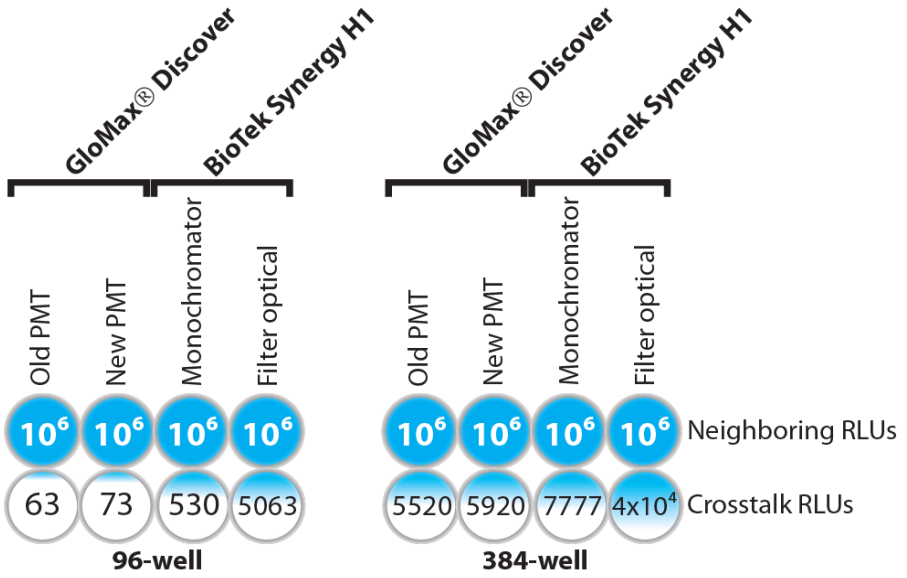
www.promega.com/discover

Performance:

- CellTox™ Green for Real-Time Cytotoxicity
- NanoBRET™ Technology for Protein Interactions
- ADP-Glo™ Kinase Selectivity Profiling
- Antibody Drug Development with ADCC Reporter Bioassays
- NanoLuc™ Luciferase Expression at Physiological Levels
- Monitoring GTPase Activity and Glucose Uptake for Oncology Research
- Nucleic Acid Quantitation with QuantiFluor Dyes

GloMax Has the Lowest Cross-talk in the Industry

The GloMax Discover has 7X less crosstalk than the BioTek H1 monochromator detector and 70X less crosstalk than the BioTek H1 filter optical detector (96 well format).



Instrument	PMT	Plate format	Blank RLUs	Avg. ATP RLUs	Avg. Water RLUs	Crosstalk (Water/ATP)	Crosstalk increase over GloMax Discover (New)
GloMax Discover	Old	96 well	300	3.99E+07	2.52E+03	6.31E-05	0.86X
	New		143	3.12E+07	2.27E+03	7.28E-05	1.00X
BioTek Synergy H1	Monochromator	96 well	25	1.05E+06	5.34E+02	5.08E-04	6.98X
	Filter Optical		20	2.99E+06	1.52E+04	5.06E-03	69.65X
GloMax Discover	Old	384 well	129	6.49E+06	3.58E+04	5.52E-03	0.93X
	New		40	6.08E+06	3.60E+04	5.92E-03	1.00X
BioTek Synergy H1	Monochromator	384 well	23	6.87E+05	5.34E+03	7.78E-03	1.31X
	Filter Optical		24	3.29E+06	1.42E+05	4.32E-02	7.31X

Streamline Your Workflow and Integrate GloMax® Systems with Hudson Robotics

Hudson Robotics

- Solo™ liquid handler
- PlateCraneEX™
- GloMax® Discover/Explorer

Controlled by Hudson Robotics SoftLinx™ software

Hudson ROBOTICS, INC.

Check the Hudson Robotics website for more details

Take-home messages

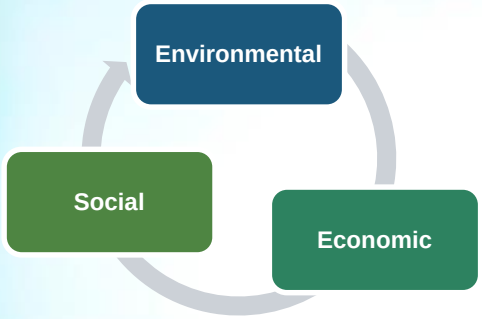
- **NanoLuc® Luciferase:**
  - Resistant to inhibitors.
  - Very bright and very small.
  - Sensitivity (poor transfection/endogenous levels).
  - Applications: Reporter gene, protein stability, protein-protein interactions, receptor-ligand, translocation, biosensors…
  - Nano-Glo® Dual-Luciferase® Assay is the most powerful dual reporter assay available.
  - Nanoluc was developed using GloMax® instruments.
- **Glomax® instruments:**
  - Broader dynamic range, better sensitivity and lower well-to-well cross-talk for more usable data from your experiment.
  - Easy to use: Simple Tablet PC touchscreen navigation with full PC capabilities and a state-of-the-art Graphical User Interface. Auto-gain adjustment.

Corporate Responsibilty

Focused on Corporate Responsibility

Improving Promega environmental and social impact

- Based on the triple bottom line framework
- Uses Global Reporting Initiative Guidelines
- Member of UN Global Compact



THE GLOBAL COMPACT

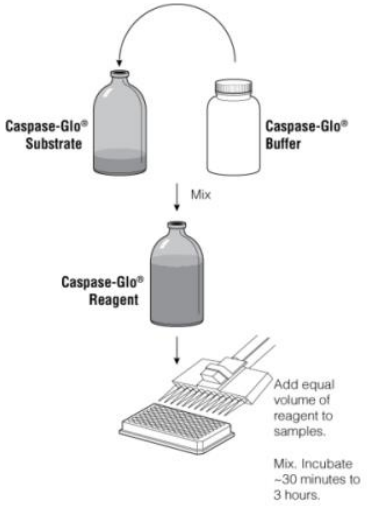
Global Reporting Initiative™

Technical Support

Promega Supports Your Entire Workflow for Reporter, Cell-Based Assays, and Quantitation

Investigate → Quantitate → Analyze

Add – Mix – Read :
Cell Viability, Apoptosis,
Gene Reporter assays,
Drug Discovery assays

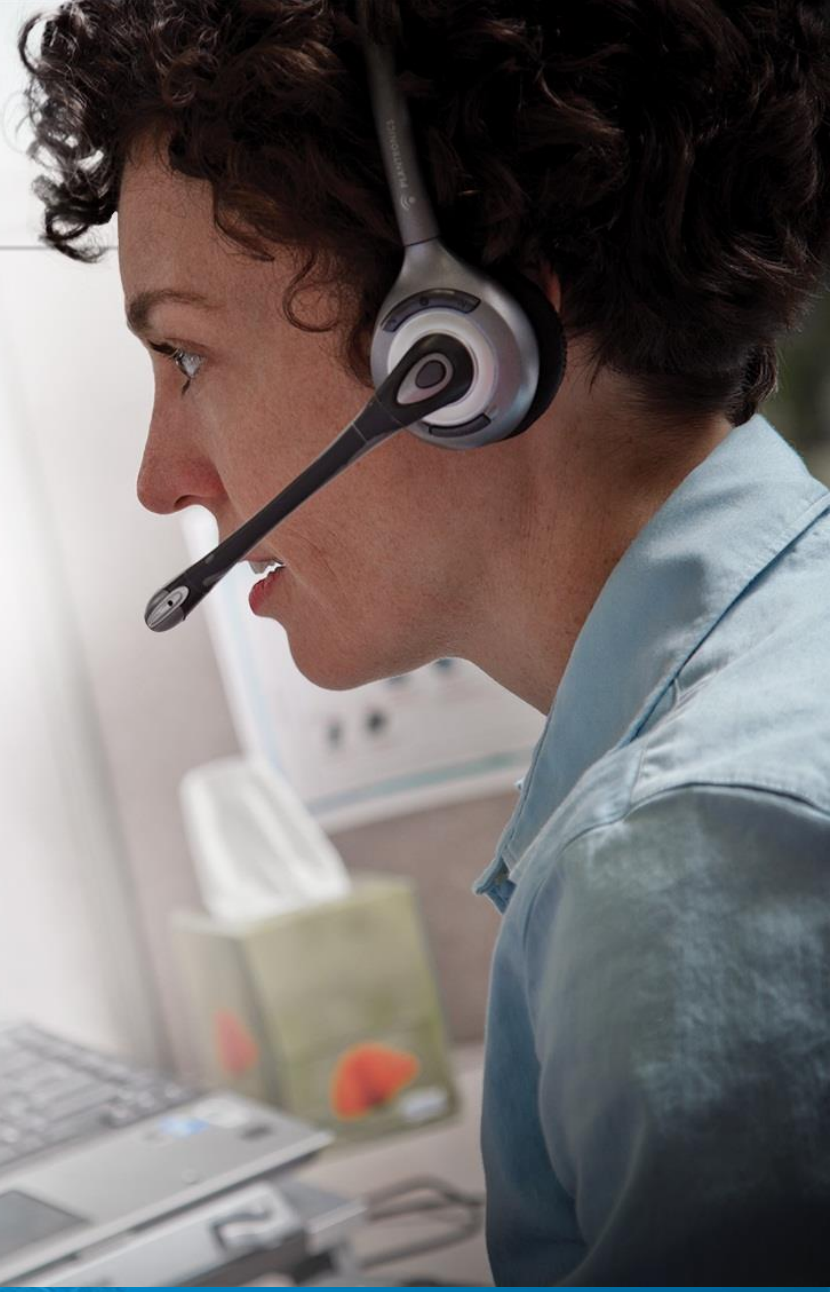


GloMax® Systems
(Discover, Explorer, Navigator)

One Call Supports it All

Scientists to help you:
- Interpret results
- Troubleshoot issues
- Design experiments

Readily Available Global Scientific Customer Support

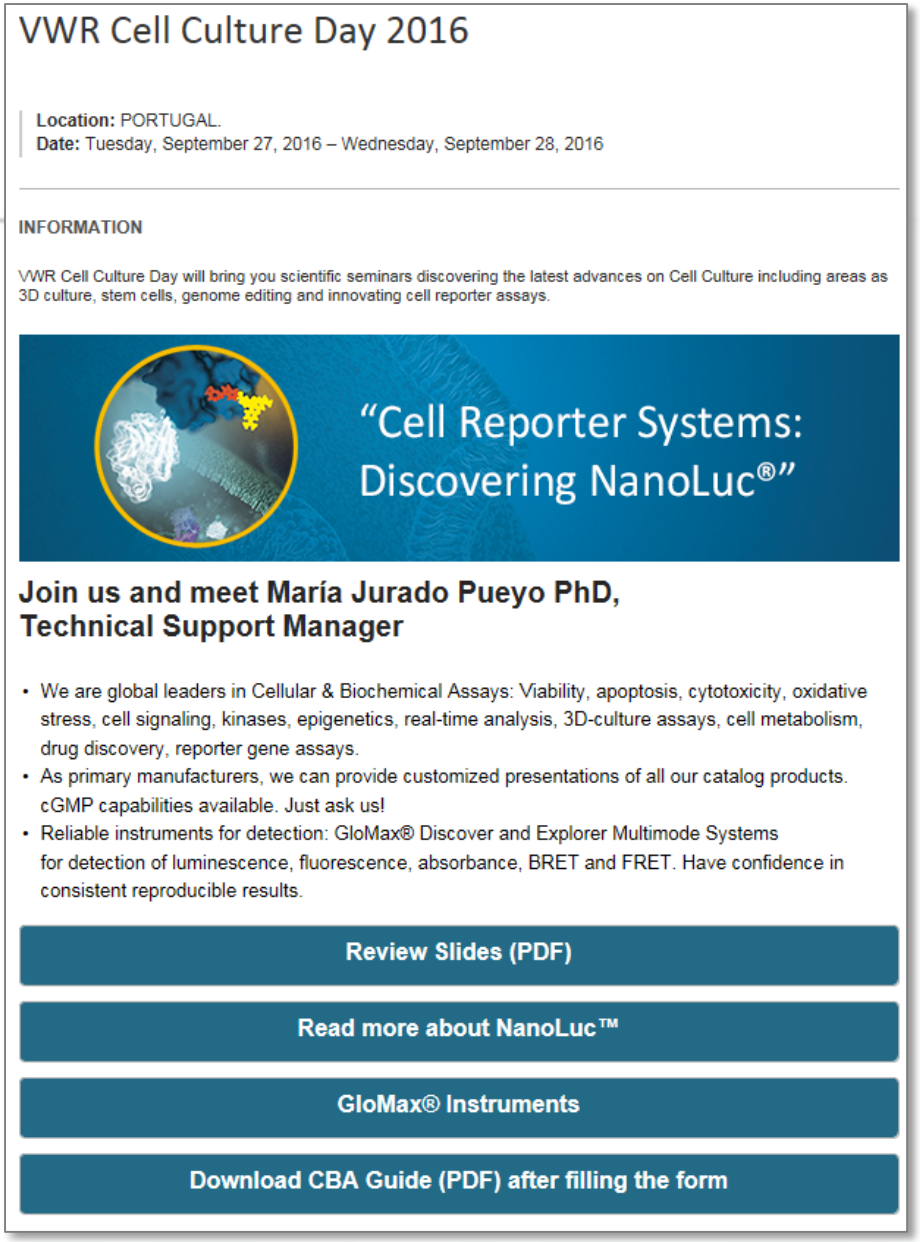


- Technical Services Scientists available by phone, e-mail or chat

- Knowledgeable sales force of scientists helping scientists
- Experienced Customer Service staff to take your orders
- Technical resources on web site including videos, protocols, technical articles and more

Visit our web!

http://bit.ly/**PromegaCellDay16**



Thanks for your attention!

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+34 91 4903901
esp_techserv@promega.com