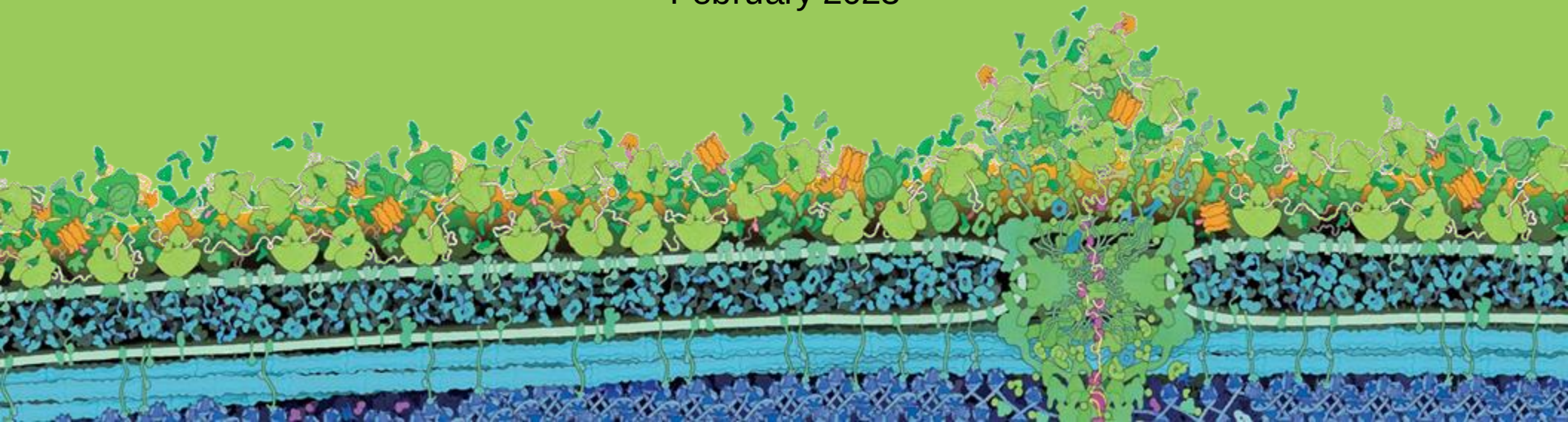
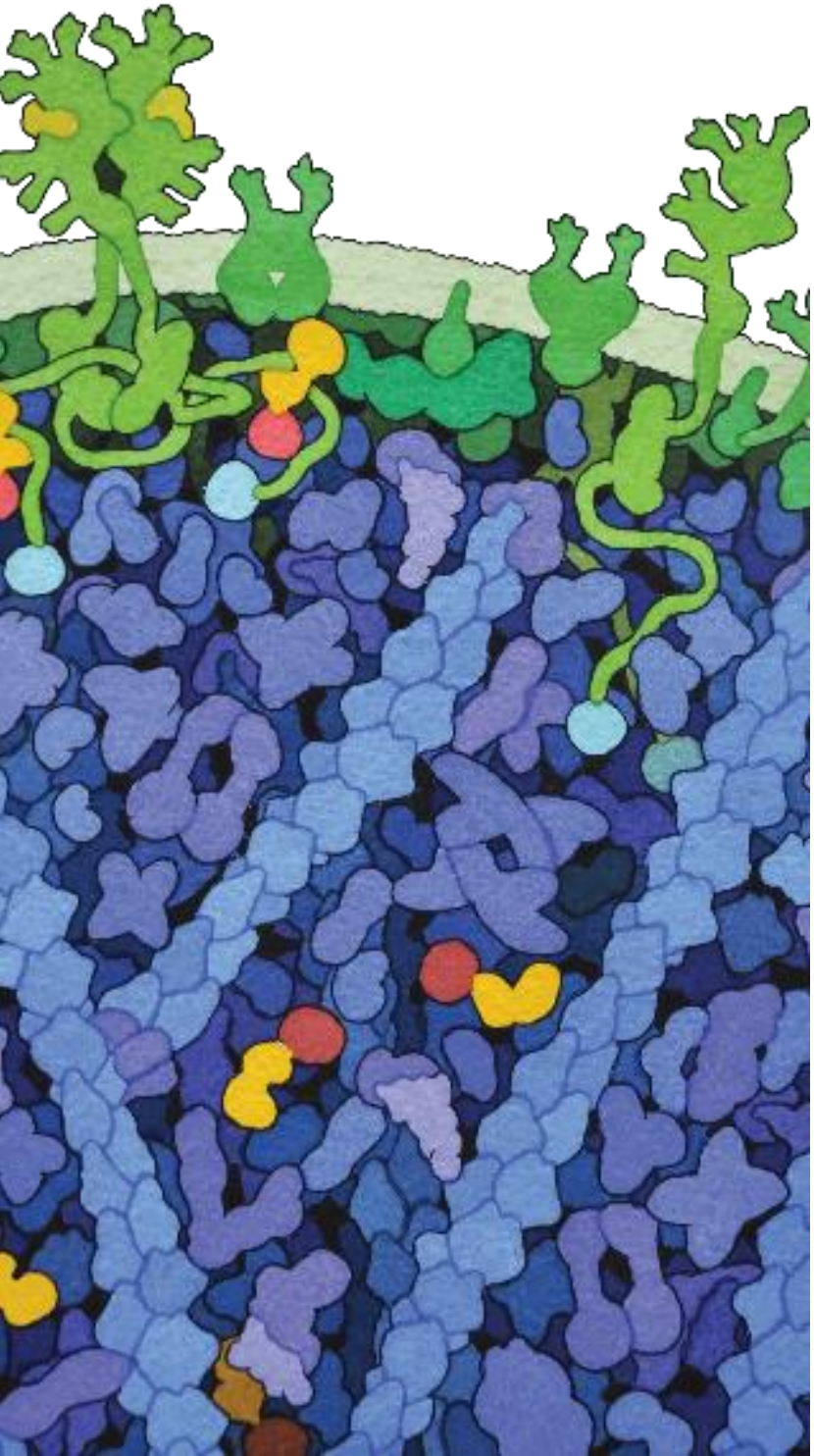
Promega

Bioluminescent Protein Reporters

Quantifying Changes in Protein Abundance ● Localization ● Interaction with NanoLuc® Technologies

February 2023





Bioluminescent Protein Reporters

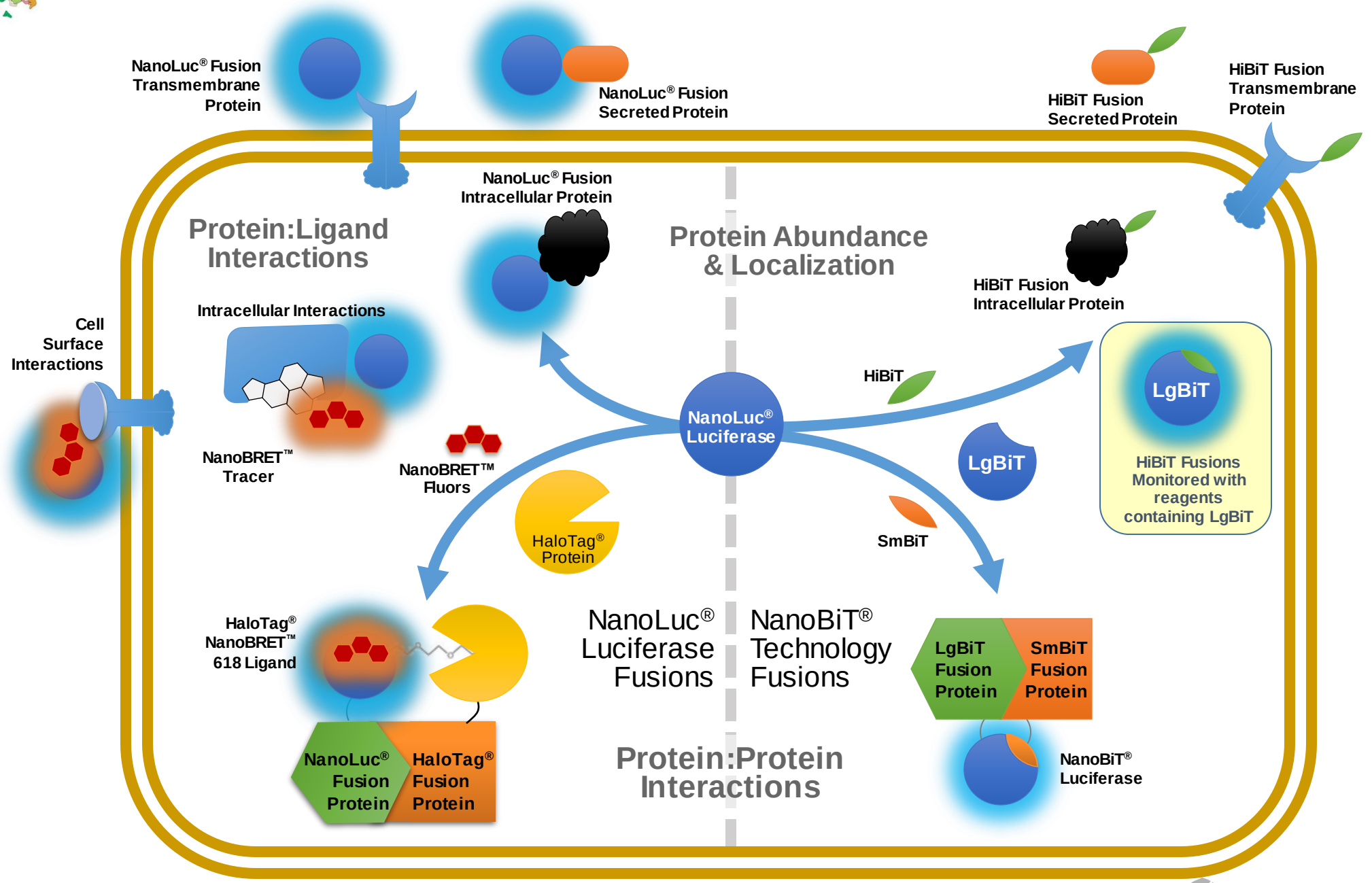
Proteins process signals from outside the cell and guide changes in the cell

Proteins can alter cells through changes in:

- Abundance
- Localization
- Modification
- Interaction

Research goals are often directed to monitoring these changes on 1 out of 20,000 proteins within the cell

Protein Reporter Applications of NanoLuc® Technologies



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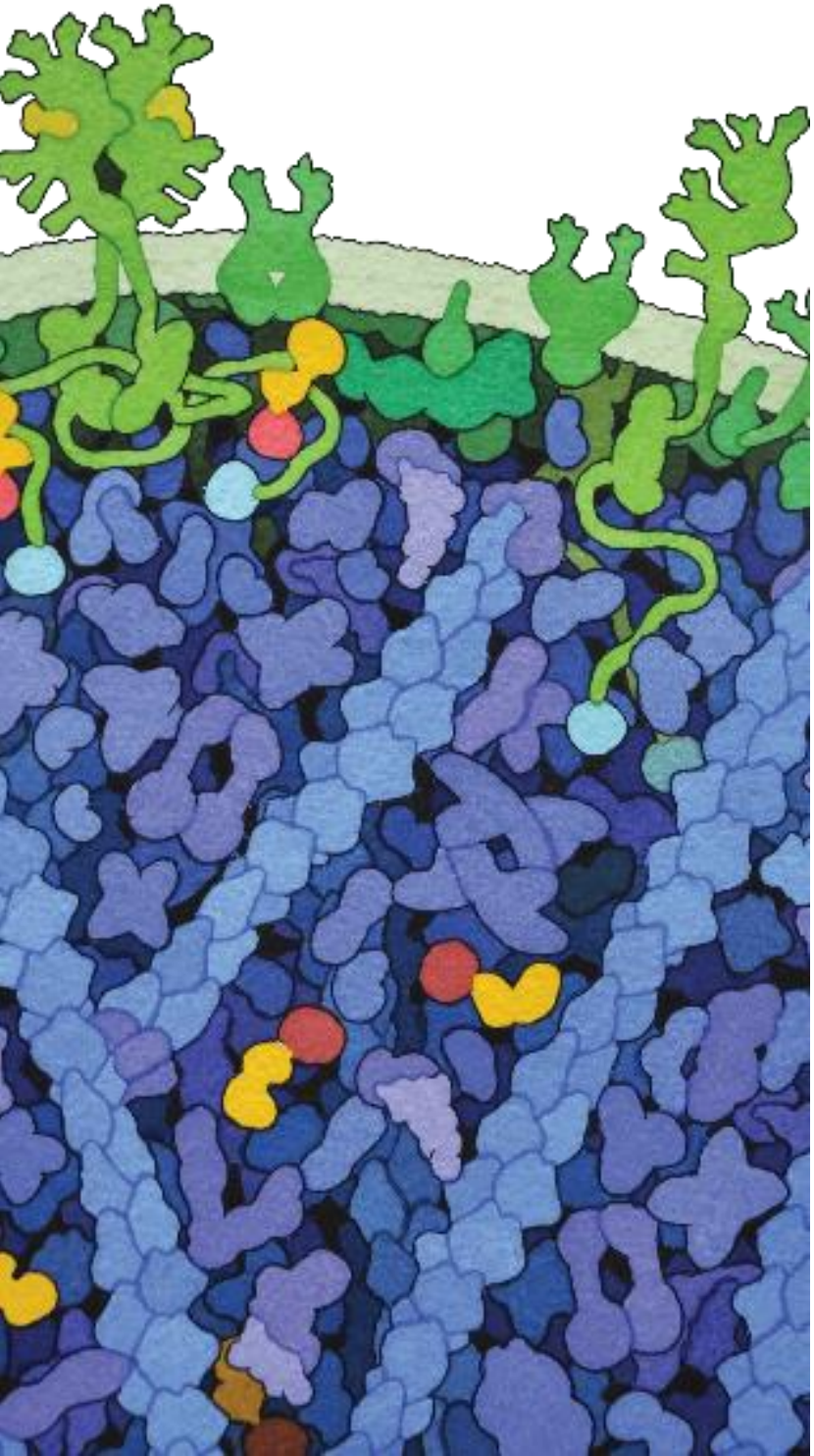
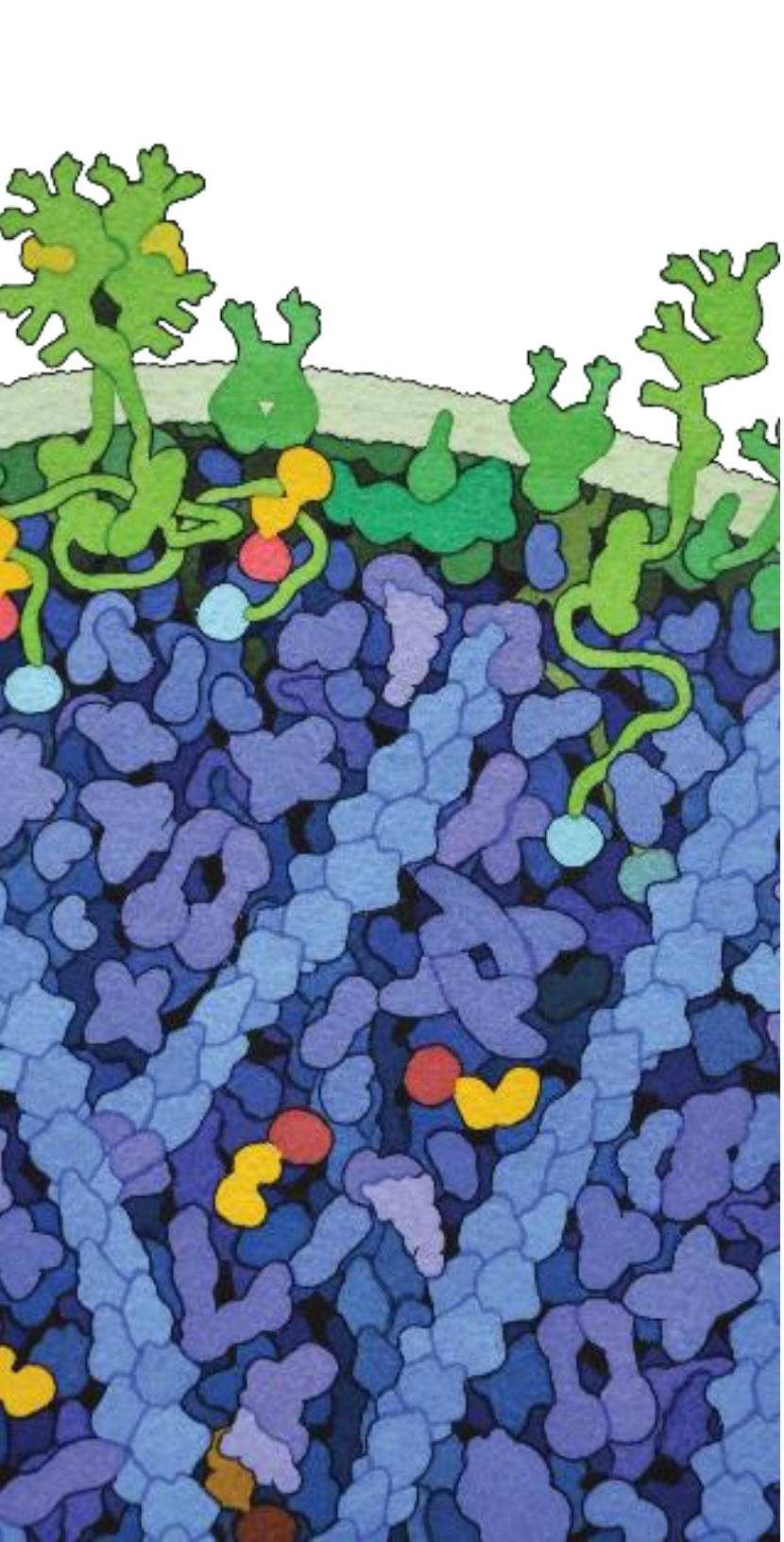


Bioluminescent Protein Reporters



Measuring Protein Abundance & Localization

NanoBiT® Structural Complementation Technology

High-affinity, spontaneous complementation subunits HiBiT and LgBiT

Nano-Glo® HiBiT Detection Systems

Protein of Interest

HiBiT Tag
(11 amino acids)

LgBiT
(17.6kDa)

in HiBiT™ Detection Reagent

NanoBiT™ Luciferase

Luminescence

Structural complementation generates a luminescent signal.

NanoLuc® Luciferase

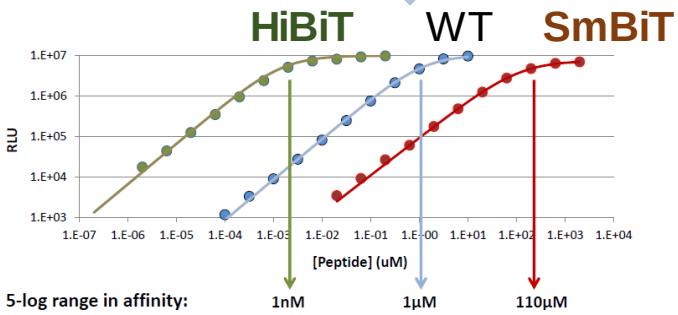
91 splice sites tested

Evolved to improve stability & expressability

LgBiT 156aa

11aa

350 sequence variants tested



High Affinity Spontaneous Complementation

Low Affinity Assisted Complementation

Read about the design and use of this assay.

Dixon, A.S., *et al.* (2016) NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem. Biol.* **11**, 400-8.

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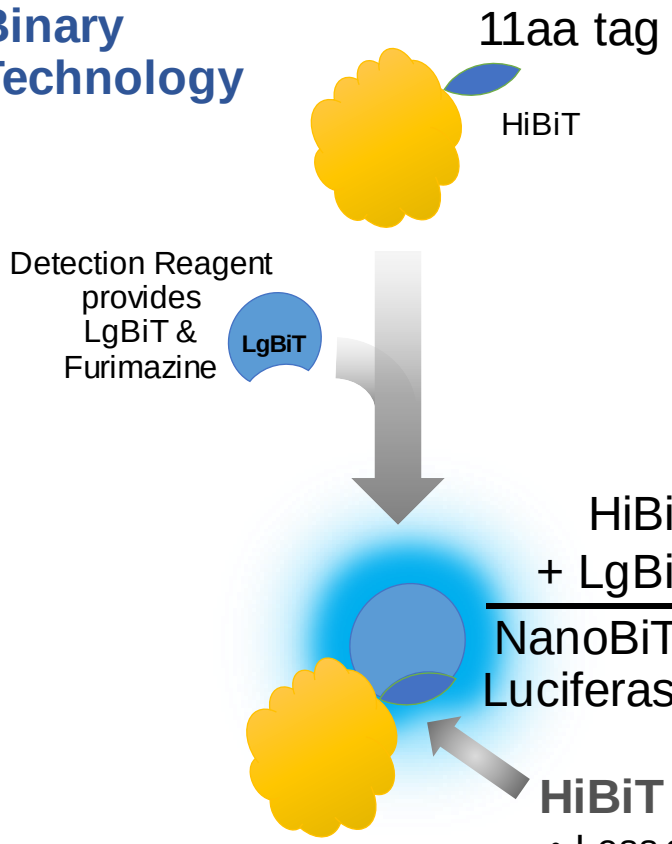
HiBiT Protein Tagging Applications

Less obtrusive and similar sensitivity to NanoLuc® Fusions

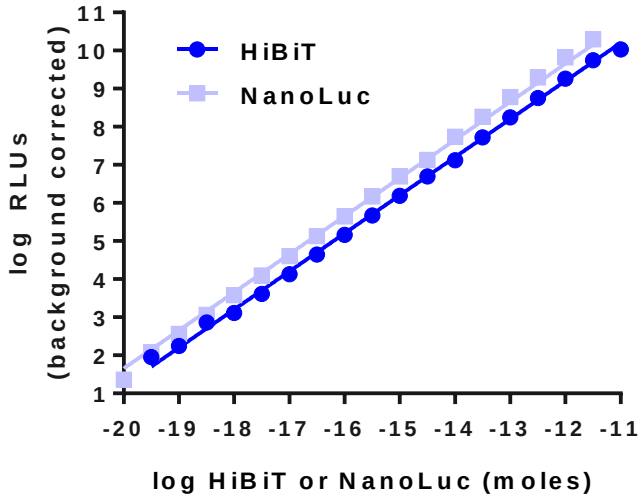
NanoLuc® Binary Technology

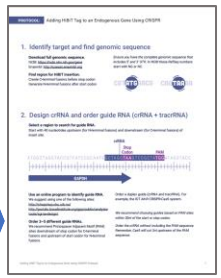


HiBiT Tagging

- Less obtrusive tag
- Simplified Cloning-free CRISPR/Cas9 Knock-In

NanoBiT® Luciferase formed from HiBiT and LgBiT subunits give sensitivity similar to NanoLuc® Luciferase





www.promega.com/hibit for CRISPR/Cas9 protocol

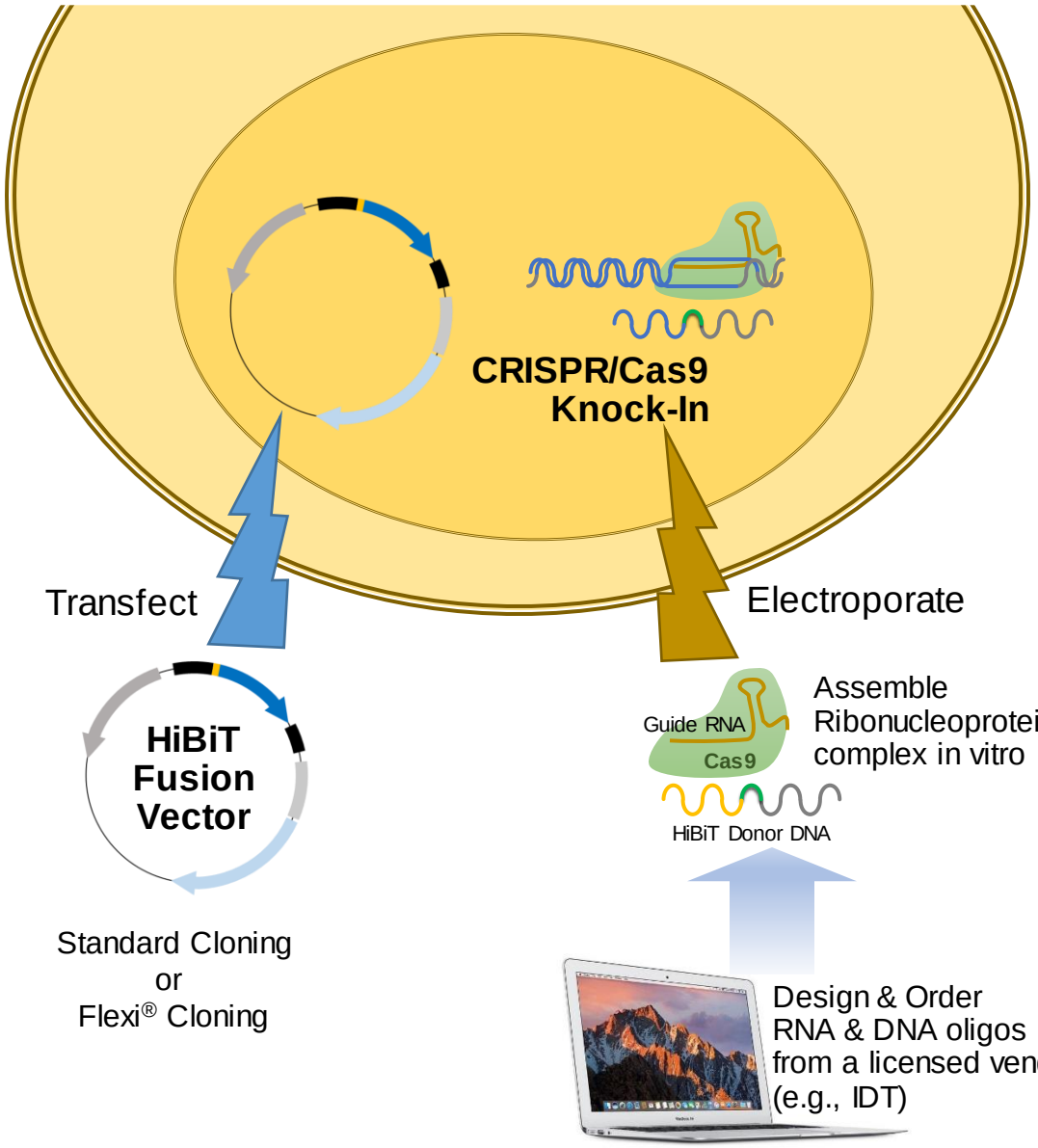
Read the papers about the design and use of this assay

Schwinn, M.K., *et al.* (2018) CRISPR-mediated tagging of endogenous protein with a luminescent peptide. *ACS Chem. Biol.* **13**, 467-74.

Schwinn, M.K., *et al.* (2020) A Simple and Scalable Strategy for Analysis of Endogenous Protein Dynamics. *Sci. Rep.* **10**, 8953.

Getting a HiBiT Tag on your Protein of Interest

HiBiT Fusion Vectors and Cloning-Free CRISPR/Cas9 Knock-In

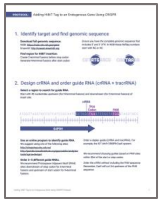


The three **pBiT3.1 HiBiT Vectors** are configured to append the 11 amino acid HiBiT peptide tag to the amino or carboxy terminus of the target protein. These vector contain a multiple cloning region to generate an in-frame HiBiT fusion protein. The pBiT3.1 Vectors can be used for both stable and transient gene expression and encode kanamycin resistance for bacterial selection and blasticidin resistance for mammalian selection. The flexible linker between the protein of interest and the HiBiT tag will vary in length, depending on the restriction enzyme used.

Product	Cat. #	Size
pBiT3.1-N [CMV/HiBiT/Blast] Vector	N2361	20µg
pBiT3.1-C [CMV/HiBiT/Blast] Vector	N2371	20µg
pBiT3.1-secN [CMV/HiBiT/Blast] Vector	N2381	20µg

The three **HiBiT CMV-neo Flexi® Vectors** are configured to facilitate simple, efficient transfer of the gene of interest into a vector designed for genetic attachment of the HiBiT peptide tag to the amino or carboxy terminus of the protein of interest using the Flexi® Cloning System (Cat.# C8640). The vectors can be used for both stable and transient gene expression and encode kanamycin resistance for bacterial selection and neomycin resistance for mammalian selection.

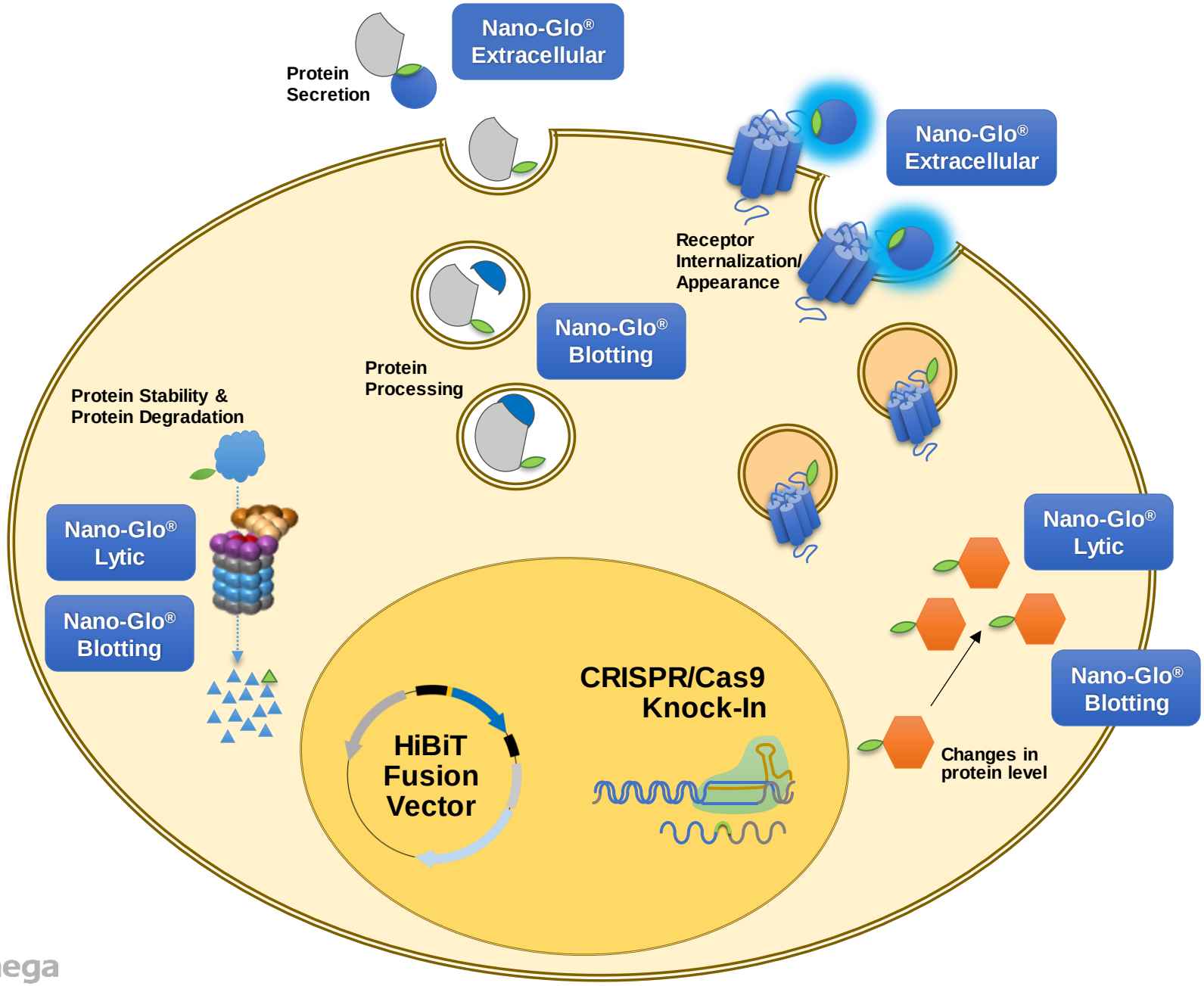
Product	Cat. #	Size
pFN38K HiBiT CMV-neo Flexi® Vector	N2401	20µg
pFC37K HiBiT CMV-neo Flexi® Vector	N2391	20µg
pFN39K secHiBiT CMV-neo Flexi® Vector	N2411	20µg



Simplified Cloning-free CRISPR/Cas9 Knock-In
www.promega.com/hibit
for CRISPR/Cas9 Guidelines

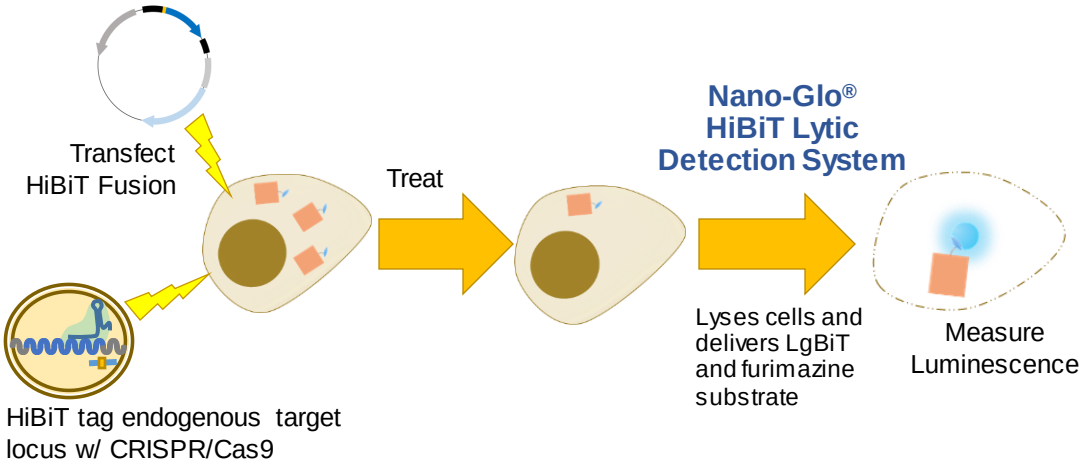
Studying Protein Dynamics through HiBiT-Tagging

Three Systems to Detect and Quantify HiBiT-tagged Proteins



Nano-Glo® HiBiT Lytic Detection System

Accurately quantify any HiBiT-tagged protein expressed in cells



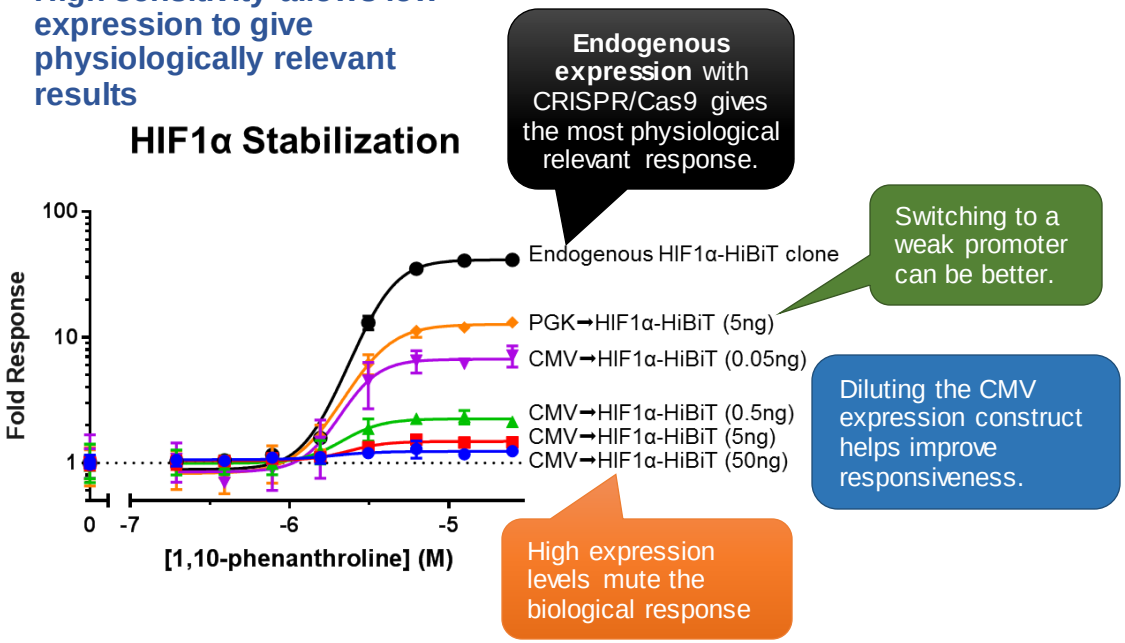
With the Nano-Glo® HiBiT Lytic Detection System, the quantity of HiBiT-tagged protein is measured directly in cell lysates with a simple add-mix-read assay. The Nano-Glo® HiBiT Detection Reagent is added to cells expressing HiBiT-tagged proteins, lysing the cells and providing the LgBiT Protein and furimazine substrate necessary for luminescence.

The high-affinity interaction between LgBiT Protein and HiBiT tag reconstitutes the luminescent NanoBiT® enzyme. This results in a highly quantitative assay with fewer processing steps compared to standard antibody-based detection methods.

- Sensitive bioluminescent protein detection
- Simple add-and-read assay—no antibodies required
- Quantitate over 7 logs of linear dynamic range

Product	Cat. #	Size
Nano-Glo® HiBiT Lytic Detection System	N3030	10ml
	N3040	100ml
	N3050	10 x 100ml

High sensitivity allows low expression to give physiologically relevant results



Read the paper about the design and use of this assay

Schwinn, M.K., *et al.* (2018) CRISPR-mediated tagging of endogenous protein with a luminescent peptide. *ACS Chem. Biol.* **13**, 467-74.

Recent Citations:

Somiya, M. and Kuroda, S. (2021) Real-Time Luminescence Assay for Cytoplasmic Cargo Delivery of Extracellular Vesicles. *Anal. Chem.* **93**, 5612-5620.

Chen, Y., *et al.* (2021) A high-throughput screen for TMPRSS2 expression identifies FDA-approved compounds that can limit SARS-CoV-2 entry. *Nat. Comm.* **12**, 3907.

Veits, G.K., *et al.* (2021) Development of an Achilles TAG degradation system and its application to control CAR-T activity. *Curr. Res. Chem. Biol.* **1**, 100010.

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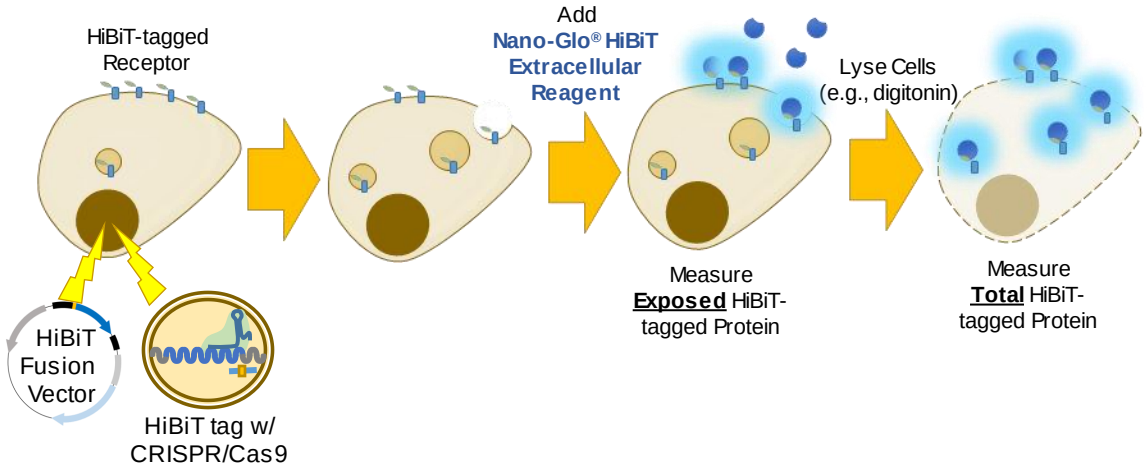
Nano-Glo® HiBiT Extracellular Detection System

Quantify HiBiT-tagged proteins expressed on/secreted from the cell



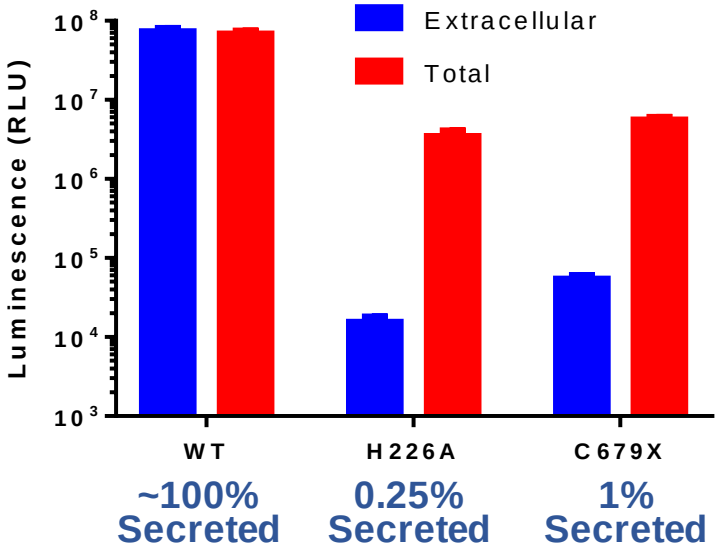
The Nano-Glo® HiBiT Extracellular Detection System quantitates cell surface or secreted protein expression in minutes using a simple add-mix-read assay format.

Using the nonlytic Nano-Glo® HiBiT Extracellular Detection Reagent, any proteins that are tagged with the 11 amino acid HiBiT peptide and expressed outside of the cell can be specifically quantitated. The detection reagent contains the complementary polypeptide LgBiT, which spontaneously interacts with the HiBiT tag to reconstitute the bright, luminescent NanoBiT® enzyme..

- Specific, live-cell detection of extracellular expressed or secreted proteins
- Simple add-and-read assay—no antibodies required
- Quantitate over 7 logs of linear dynamic range

Product	Cat. #	Size
Nano-Glo® HiBiT Extracellular Detection System	N2420	10ml
	N2421	100ml
	N2422	10 x 100ml

PCSK9-HiBiT Extracellular and Lytic Signals



Read the paper about the design and use of this assay

Schwinn, M.K., *et al.* (2018) CRISPR-mediated tagging of endogenous protein with a luminescent peptide. *ACS Chem. Biol.* **13**, 467-74.

Recent Citations:

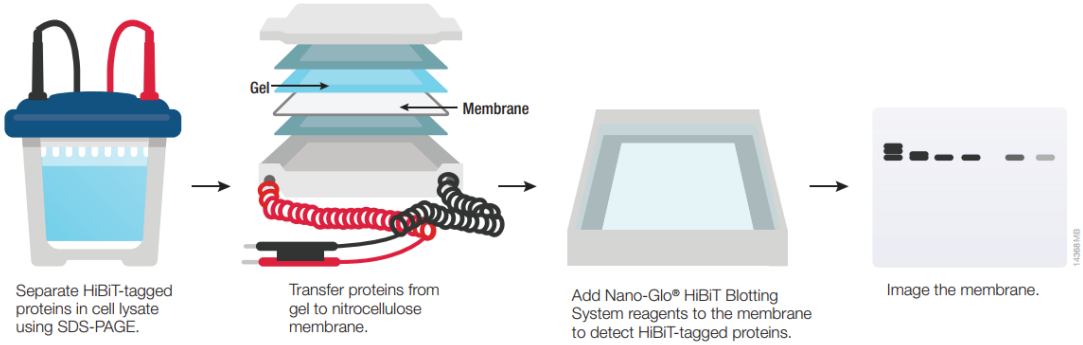
Yamada, K., *et al.* (2022) Extended-synaptotagmin 1 engages in unconventional protein secretion mediated via SEC22B+ vesicle pathway in liver cancer. *PNAS* **119**, e2202730119.

Liu, J., *et al.* (2021) A variant *ECE1* allele contributes to reduced pathogenicity of *Candida albicans* during vulvovaginal candidiasis. *PLoS Pathog.* **17**, e1009884.

Winter, J., *et al.* (2021) Chronic oxytocin-driven alternative splicing of Crfr2α induces anxiety. *Mol. Psychiatry.*

Nano-Glo® HiBiT Blotting System

Fast luminescent detection of HiBiT-tagged proteins



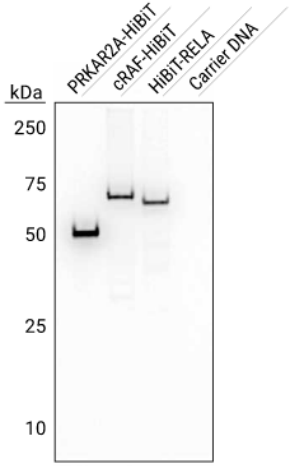
Using the Nano-Glo® HiBiT Blotting System, any HiBiT-tagged proteins can be visualized on membranes following gel separation. The blotting reagent, which contains the LgBiT Protein and furimazine substrate, is added directly to the membrane, and a luminescent signal is produced only where HiBiT is present.

This simple method requires as few as 5 minutes to perform in contrast to the multiple hours and many steps needed for standard antibody-based blotting protocols. Because luminescence is only produced only where HiBiT is present, background is minimal...

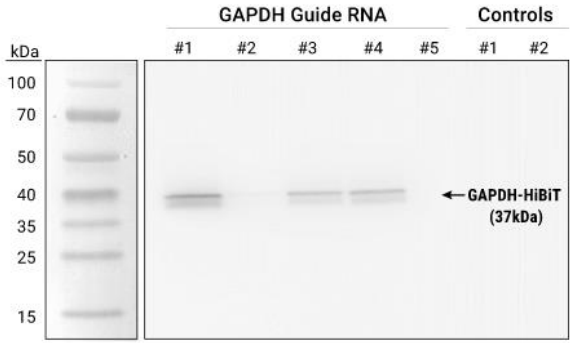
- Determine protein size and quantify expression on blots
- Protocol requires only minutes, with few processing steps
- Femtogram sensitivity proportional over five orders of magnitude

Product	Cat. #	Size
Nano-Glo® HiBiT Blotting System	N2410	100ml
HiBiT Control Protein (20µM)	N3010	100µl

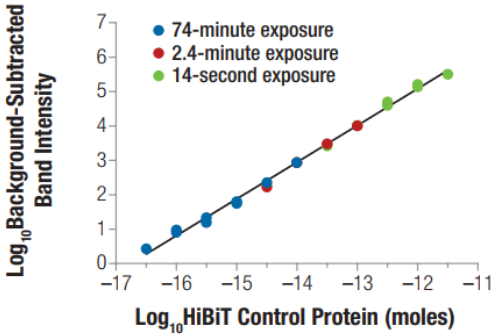
Measure transiently expressed HiBiT-tagged proteins



Measure endogenously-HiBiT-tagged proteins



Signal Proportional Over 5 Orders of Magnitude



Read the paper about the design and use of this assay

Schwinn, M.K., *et al.* (2018) CRISPR-mediated tagging of endogenous protein with a luminescent peptide. *ACS Chem. Biol.* **13**, 467-74.

Recent Citations:

Balazova, L., *et al.* (2021) GPR180 is a component of TGFβ signaling that promotes thermogenic adipocyte function and mediates the metabolic effects of the adipocyte-secreted factor CTHRC1. *Nat. Comm.* **12**, 7144.

Challa, K., *et al.* (2021) Damage-induced chromatome dynamics link Ubiquitin ligase and proteasome recruitment to histone loss and efficient DNA repair. *Mol. Cell* **81**, 811-829.e6.

Loughran, G., *et al.* (2020) Unusually efficient CUG initiation of an overlapping reading frame in POLG mRNA yields novel protein POLGARF. *PNAS* **117**, 24936-24946.

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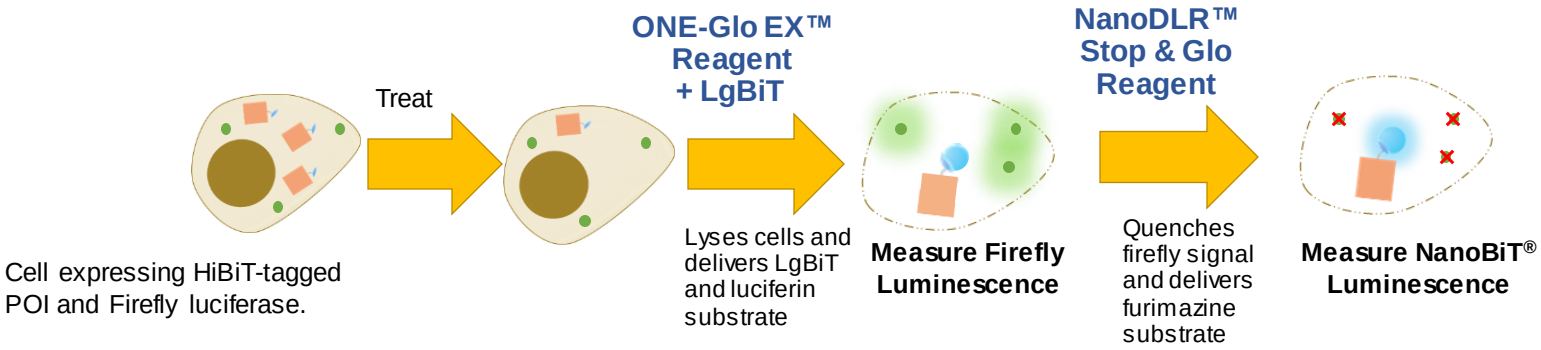
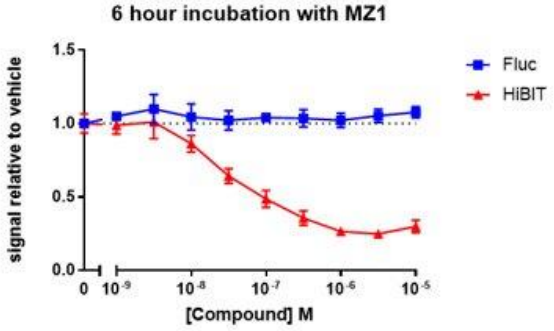
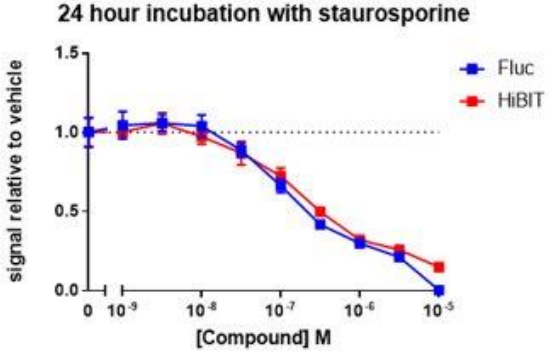
Nano-Glo® HiBiT Dual-Luciferase Reporter System

Measure Firefly and HiBiT signal in the same well





HiBiT-specific effects with treatment. Firefly remains constant.



Non-specific effects with treatment. Firefly and HiBiT signal decrease.

The Nano-Glo® HiBiT Dual-Luciferase® Reporter System (HiBiT NanoDLR™) allows for sequential measurement of firefly luciferase (Fluc) and HiBiT-tagged proteins from the same sample in an "add-read-add-read" assay format. Fluc is used as a constitutively expressed control to indicate non-specific compound effects on cell viability or protein expression, rather than specific changes in the levels of the HiBiT-tagged protein of interest, aiding in the interpretation of protein modulation screens and studies.

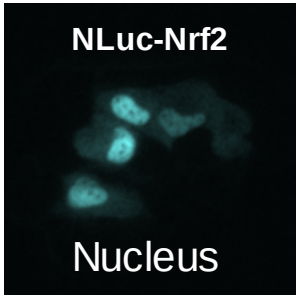
The 11-amino acid HiBiT peptide tag can be added to a protein of interest using CRISPR/Cas9 gene editing coupled with ectopic expression of Fluc. Alternatively, use a bicistronic HiBiT entry vector to express both the HiBiT-protein fusion and constitutive Fluc on the same mRNA.

Product	Cat. #	Size
Nano-Glo® HiBiT Dual-Luciferase® Reporter System	CS1956A08	10ml
	CS1956A09	100ml
Nano-Glo® HiBiT Dual-Luciferase® Bicistronic Vectors	CS1956B08 -13	20µg

HiBiT DLR and the bicistronic HiBiT entry vectors are Early Access Materials. Please contact your local Promega Representative or TailoredSolutions@promega.com to order.

NanoLuc® Fusion Vectors
Generate N- or C-Terminal Fusions to NanoLuc® Luciferase

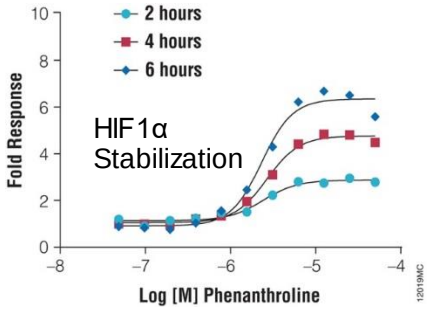
Localization/ Trafficking



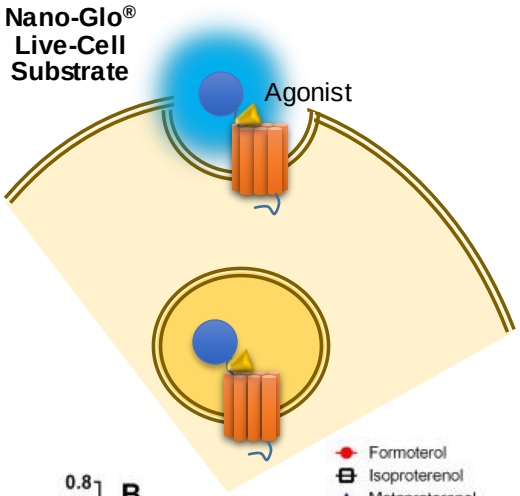
Olympus LV200

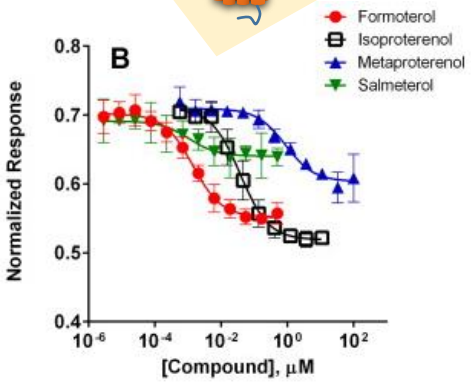
Hall, M.P., et al. (2012) *ACS Chem Biol* **7**:1848

Stability/ Abundance



Endocytosis





Robers, M.B., *et al.* (2015) A luminescent assay for real-time measurements of receptor endocytosis in living cells. *Anal. Biochem*. **489**, 1-8.

The **pNLF1 Vectors** use traditional cloning with a multiple cloning site (MCS) to generate N- or C-terminal fusions to NanoLuc® luciferase. Create full-length Nluc protein fusions using the pNLF1-N [CMV/Hygro] Vector (N terminus) and pNLF1-C [CMV/Hygro] Vector (C terminus). In addition, attach secreted Nluc to the N terminus of the protein of interest using the pNLF1-secN [CMV/Hygro] Vector. All vectors contain a mammalian selectable marker to create a stable line.

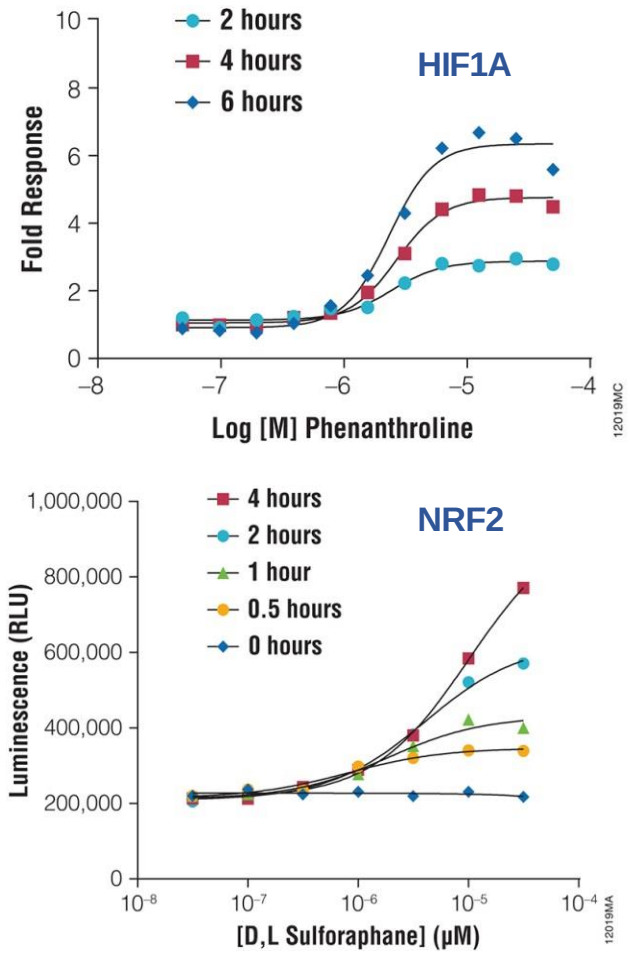
Product	Cat. #	Size
pNLF1-N [CMV/Hygro] Vector	N1351	20µg
pNLF1-C [CMV/Hygro] Vector	N1361	20µg
pNLF1-secN [CMV/Hygro] Vector	N1371	20µg

Generate N-terminal fusions with NanoLuc® luciferase using the **pFN31A and pFN31K Nluc CMV-neo Flexi® Vectors** while the **pFC32A and pFC32K Nluc CMV-neo Flexi® Vectors** create C-terminal Nluc fusion proteins. These vectors are part of the Flexi® Vector Cloning System (Cat.# C8640) that uses a directional cloning method based on two rare-cutting restriction enzymes, SgfI and PmeI, that provides a rapid, efficient and high-fidelity way to transfer protein-coding regions between a variety of Flexi® Vectors without the need to resequence. All vectors contain a mammalian selectable marker to create a stable line. Please note, Flexi® vectors cannot be grown in common laboratory *E. coli* strains until an insert has been cloned into the vector.

Product	Cat. #	Size
pFN31A Nluc CMV-Hygro Flexi® Vector	N1311	20µg
pFN31K Nluc CMV-neo Flexi® Vector	N1321	20µg
pFC32A Nluc CMV-Hygro Flexi® Vector	N1331	20µg
pFC32K Nluc CMV-neo Flexi® Vector	N1341	20µg

NanoLuc® Stability Sensors

Vectors to measure intracellular protein turnover of HIF1A and NRF2



Protein stabilization and accumulation can be quantified with NanoLuc® fusion proteins. HCT-116 cells were transiently transfected with the pNLF1-HIF1α [CMV/neo] Vector and Transfection Carrier DNA (top) or the pNLF1-NRF2 [CMV/neo] Vector and pKEAP1 DNA (bottom), treated as indicated, and NanoLuc® detected using **Nano-Glo® Luciferase Assay** at the indicated time points.

The rate of protein turnover is tightly regulated for many signaling proteins involved in oncogenesis and response to cellular stress. Protein stabilization and subsequent accumulation occurs in response to changing cellular conditions resulting in activation of downstream transcriptional events. The **NanoLuc® Stability Sensors** are ready-to-use vector systems that utilize the advantages of the NanoLuc® luciferase reporter to enable stability studies of two key signaling proteins, HIF1A and NRF2, providing a method to directly measure this primary signaling event.

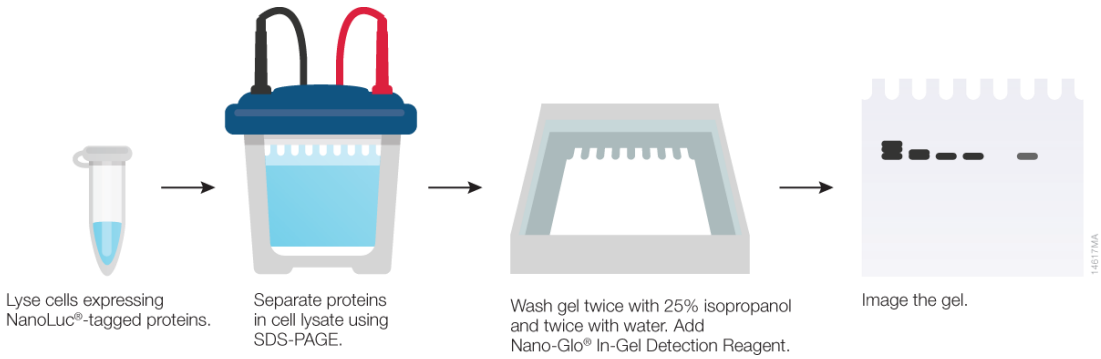
- Ready-to-use constructs are predesigned, optimized and tested for low endotoxin levels
- C-terminal fusion of HIF1A NanoLuc® luciferase reporter in pNLF1-HIF1A [CMV/neo] Vector
- NRF2 vector system has a pKEAP1-expressing vector for regulating intracellular NRF2 levels and pNLF1-NRF2 [CMV/neo] Vector expressing a C-terminal fusion with NanoLuc® luciferase

Product	Cat. #	Size
pNLF1-HIF1A [CMV/neo] Vector • pNLF1-HIF1A [CMV.neo] Vector (20µg) • Transfection Carrier DNA (20µg)	N1381	20µg
pNLF1-NRF2 [CMV/neo] Vector • pNLF1-NRF2 [CMV/neo] Vector (20µg) • pKEAP1[CMV/Hygro] Vector (20µg) • Transfection Carrier DNA (20µg)	N1391	20µg

pNLF1-NRF2 Citation:

Jiang, X., *et al.* (2020) Nuclear Factor Erythroid 2 Related Factor 2 Activator JC-5411 Inhibits Atherosclerosis Through Suppression of Inflammation and Regulation of Lipid Metabolism. *Front. Pharmacol.* **11**, 532568.

Nano-Glo® In-Gel Detection System
Directly detect NanoLuc® Fusion Proteins in polyacrylamide gels



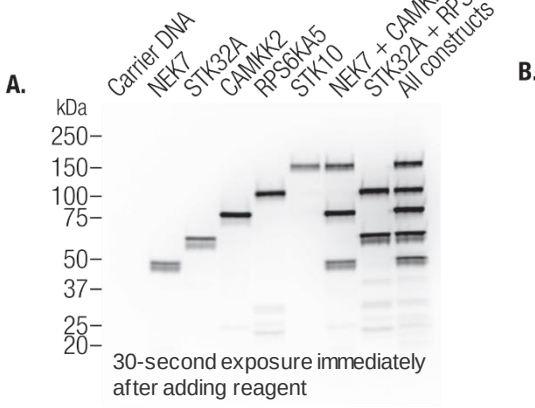
Eliminate the need for immunoblotting to detect NanoLuc® fusion proteins separated by polyacrylamide gel electrophoresis. Directly image gels after incubation with the Nano-Glo® In-Gel Detection Reagent. For native PAGE, the gels can be incubated with detection reagent and imaged in less than 15 minutes. For denaturing SDS-PAGE, two washes with 25% isopropanol followed by two washes in water are needed to remove the SDS and allow the NanoLuc® luciferase to refold before detection.

The sensitivity of NanoLuc® luciferase means proteins do not need to be overexpressed to be visualized by the Nano-Glo® In-Gel Detection System..

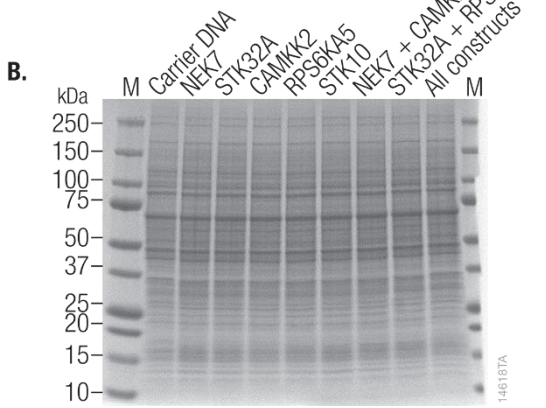
- Simply incubate native or SDS denaturing gels with reagent and image
- No transfer to membranes required for detection
- Eliminates the need for blocking or antibodies

Product	Cat. #	Size
Nano-Glo® In-Gel Detection System	N3020	100ml

NanoLuc® Fusions visualized using the Nano-Glo® In-Gel Detection System



Same gel stained with Coomassie Blue



HEK293 cells were transiently transfected individually or in combinations with five different fusions of NanoLuc® luciferase to protein kinases: NEK7, STK32A, CAMKK2, RPS6KA5 and STK10. CMV expression constructs were diluted 100-fold into carrier DNA to lower expression levels. After overnight expression, gel electrophoresis was performed using 10µl of cell lysate.

Recent Citations:

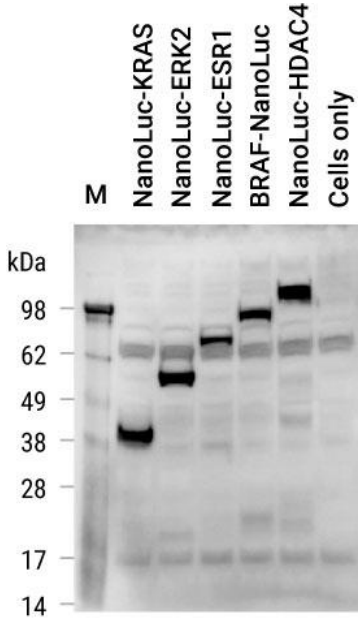
Casalino, E., *et al.* (2022) A novel high-throughput screening strategy for targeting alpha-synuclein and other long-lived proteins. *SLAS Discov.* **27**, 349-357.

Bonsergent, E., *et al.* (2021) Quantitative characterization of extracellular vesicle uptake and content delivery within mammalian cells. *Nat. Comm.* **12**, 1864.

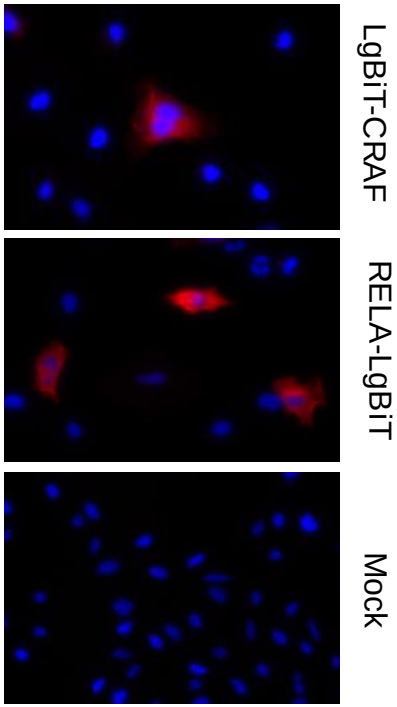
Promega

Monoclonal Antibodies for NanoLuc®, LgBiT and HiBiT

Detect NanoLuc® fusion or LgBiT- or HiBiT-tagged proteins using traditional methods



Western blot of NanoLuc® Fusions expressed in HEK293 cells.



Immunofluorescent staining of LgBiT Fusions expressed in HEK293 cells.

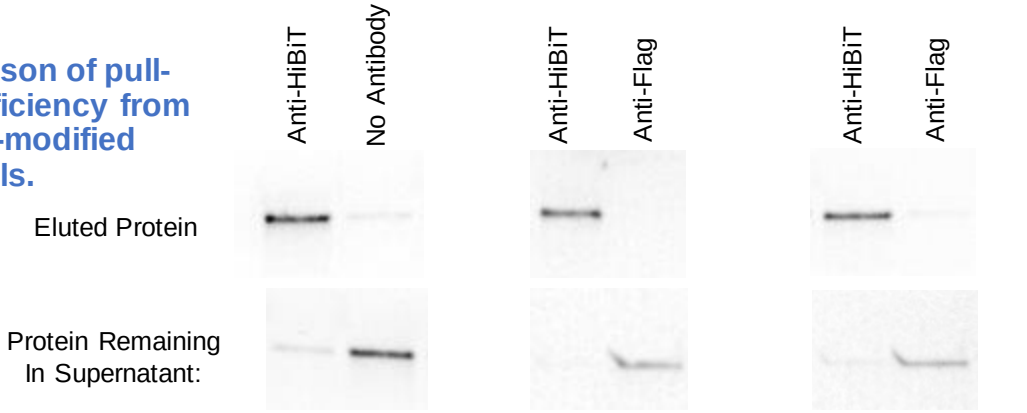
The Anti-NanoLuc® and Anti-LgBiT Monoclonal Antibodies can be used to detect NanoLuc® Luciferase or NanoLuc® fusion proteins and LgBiT fusion proteins, respectively, via western blot or immunofluorescent staining.

The Anti-HiBiT Monoclonal Antibody has been validated for western blot, immunofluorescent staining, immunoprecipitation, and FACS analysis.

These antibodies expand the toolbox associated with our NanoLuc® and NanoBiT® technologies beyond luminescence to compatibility with traditional methods.

Product	Cat. #	Size
Anti-NanoLuc® Monoclonal Antibody	N7000	100µg
Anti-LgBiT Monoclonal Antibody	N7100	100µg
Mouse Anti-HiBiT mAb (Clone 30E5)	CS2006A01	100µg
Anti-HiBiT mAb is an Early Access Material. Please contact your local Promega Representative or TailoredSolutions@promega.com to order.		

Comparison of pull-down efficiency from CRISPR-modified HeLa cells.



Recent Citations (Anti-NanoLuc®):

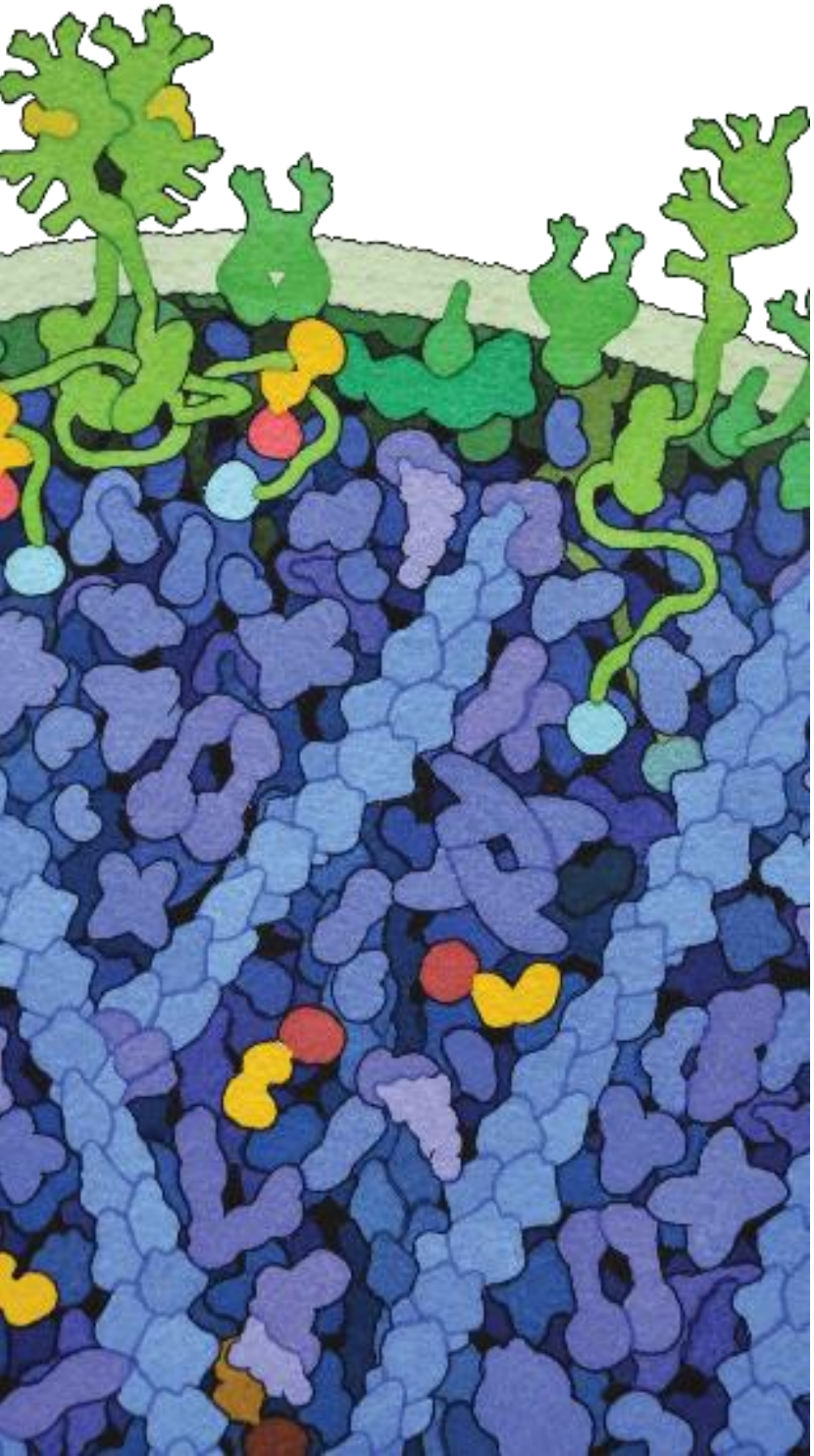
Liang, X., *et al.* (2022) Extracellular vesicles engineered to bind albumin demonstrate extended circulation time and lymph node accumulation in mouse models. *J. Extracell. Vesicles* **11**, e12248.

Recent Citations (Anti-LgBiT):

Madsen, M.S., *et al.* PPARγ lipodystrophy mutants reveal intermolecular interactions required for enhancer activation. *Nat. Commun.* **13**, 7090.

Recent Citations (Anti-HiBiT):

Seiler, K., *et al.* (2022) Hexokinase 3 enhances myeloid cell survival via non-glycolytic functions. *Cell Death Dis.* **13**, 448.

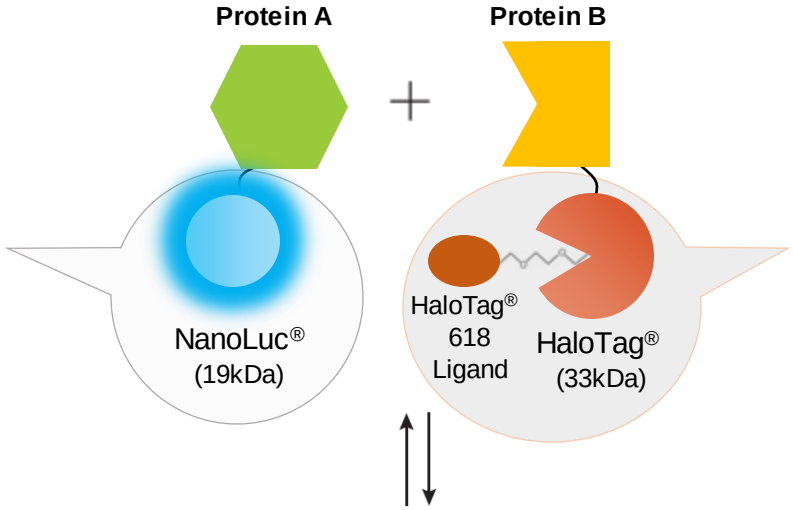


Measuring Protein:Protein Interactions

NanoBRET™ Technology

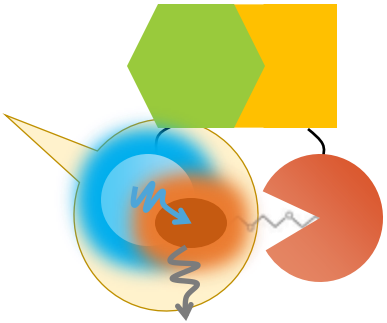
Study Protein Interaction Dynamics in Natural Cellular Context

30-fold brighter than any other luciferase donor used for BRET, NanoLuc® Luciferase fuels greater energy transfer.



Use of HaloTag® Fusion Protein and a HaloTag® Ligand as the Acceptor allowed design of the ideal fluor for use with NanoLuc® Luciferase

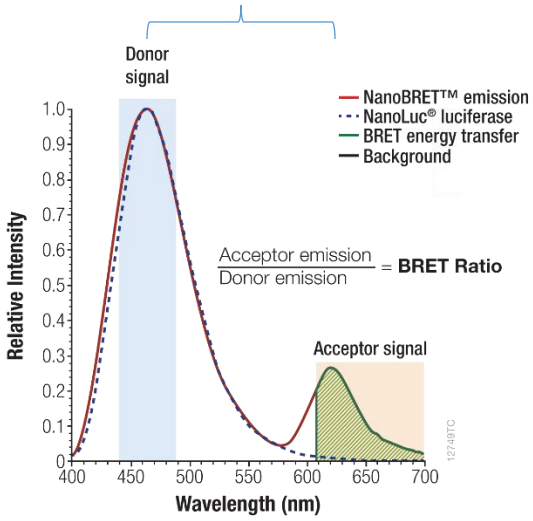
Use of a fluor rather than a fluorescent protein as the Acceptor means the NanoBRET™ Technology can be applied to Protein:Protein as well as Protein:Ligand interactions.



Fluorescence
NanoBRET™

For NanoBRET™ Transfer to occur, NanoLuc® Luciferase and HaloTag® 618 Ligand just need to be in close proximity (≤10nm).

NanoBRET™ Technology provides a large >150nm window between donor & acceptor signals for greater resolution.



GloMax® Discover is pre-optimized for reading NanoBRET™ Donor and Acceptor emissions.

Read the paper about the design and use of this assay

Machleidt, T., *et al.* (2015) NanoBRET–A novel BRET platform for the analysis of protein-protein interactions. *ACS Chem. Biol.* **10**, 1797–1804

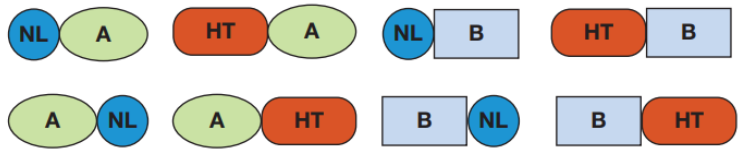
Promega

NanoBRET™ PPI Starter Systems

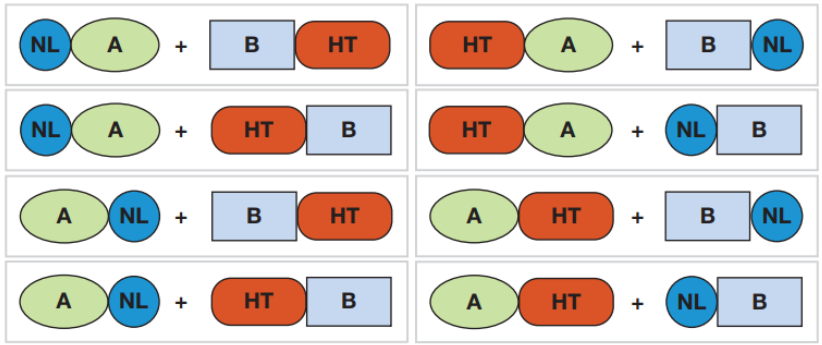
NanoLuc® and HaloTag® Fusion Vectors Sets & Detection Reagents

Generating and optimizing a NanoBRET™ assay

1. **Generate clones** by appending NanoLuc® (NL) donor and HaloTag® (HT) acceptor tags to protein A and protein B (up to 8 possible clones).



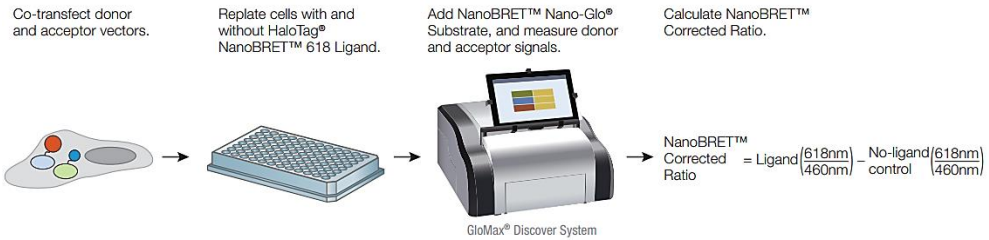
2. **Test combinations** to find the best energy transfer (up to 8 possible combinations).



3. **Optimize transfection** of the best combinations for optimal donor to acceptor DNA ratio to minimize unbound donor and maximize dynamic range.

4. **Validate** using an available inhibitor or activator to test specific response with optimized transfection condition. Alternatively, test specificity using saturation assays.

NanoBRET™ Assay Workflow



The **NanoBRET™ PPI Starter Systems** provide the vectors required to create NanoLuc® Luciferase and HaloTag® protein fusions to target proteins of interest, a Positive Control Pair (p53, MDM2) and the **NanoBRET™ Nano-Glo® Detection Systems** (200 rxn), which contains the substrate used by NanoLuc® Luciferase to generate the donor signal and the HaloTag® NanoBRET™ 618 Ligand for the fluorescent energy acceptor.

- Live-cell reagents allow you to detect protein:protein interactions in real time using full-length proteins or fragments.
- Reversible assay technology allows you to study both induction and inhibition of protein interactions.
- Assays can be performed in 96- or 384-well formats with low variability and high reproducibility.
- Standard detection provides the brightest signal for timepoints <2 hours; kinetic detection is best for timepoints 2-72 hours

Product	Cat. #	Size
NanoBRET™ PPI Assay MCS Starter System	N1811	Each
NanoBRET™ PPI Assay Flexi Starter System	N1821	Each
NanoBRET™ Nano-Glo® Standard Detection System	N1661	200 rxn
	N1662	1000 rxn
	N1663	10,000 rxn
NanoBRET™ Nano-Glo® Kinetic Detection System	N2583	200 rxn
	N2584	1000 rxn
	N2585	10,000 rxn

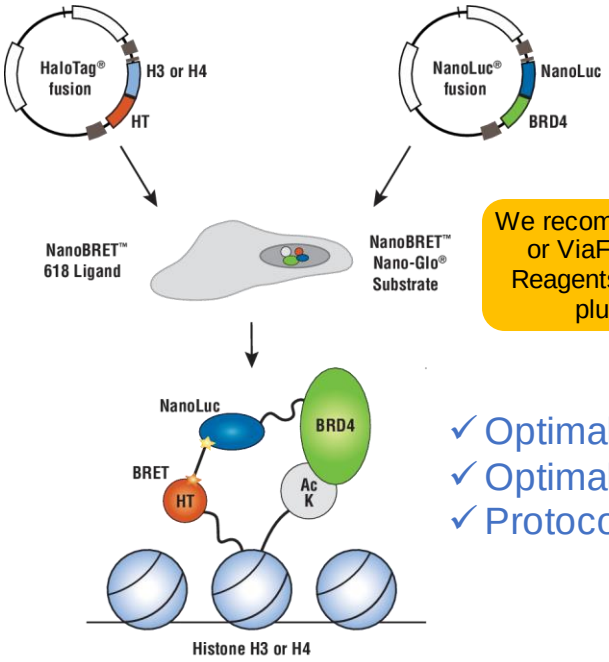
Recent Citations:

Ganier, L., *et al.* (2022) Discovery of Small-Molecule Inhibitors of the PTK7/β-Catenin Interaction Targeting the Wnt Signaling Pathway in Colorectal Cancer. *ACS Chem. Biol.* **17**, 1061-1072.

Di Mauro, V., *et al.* (2021) Peptide-Based Targeting of the L-Type Calcium Channel Corrects the Loss-of-Function Phenotype of Two Novel Mutations of the *CACNA1* Gene Associated with Brugada Syndrome. *Front. Physiol.* **11**, 616819.

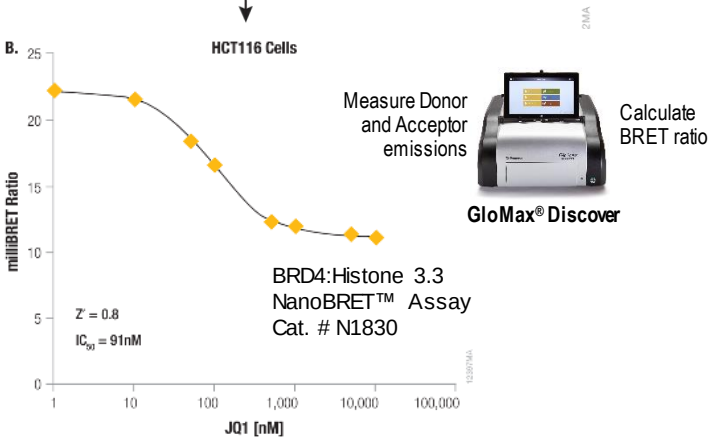
Lim, W.F., *et al.* (2021) Gene therapy with AR isoform 2 rescues spinal and bulbar muscular atrophy phenotype by modulating AR transcriptional activity. *Sci. Adv.* **7**, eabi6869.

NanoBRET™ Prebuilt PPI Assays
Predesigned, Optimized Assays



We recommend FuGENE® HD or ViaFect™ Transfection Reagents for max efficiency plus low toxicity.

- ✓ Optimal orientation determined
- ✓ Optimal transfection ratio found
- ✓ Protocol included



Dose-dependent decrease in BRET ratio by the bromodomain inhibitor, JQ1.

The predesigned **NanoBRET™ PPI Assays** enable interaction studies of various full-length proteins or protein fragments. These are ready-to-use vectors with no need to optimize tag orientation or transfection ratio. Live-cell reagents allow you to detect protein:protein interactions in real time.

- Detect protein interactions in live cells, even at low expression levels
- Choose from prebuilt or custom assay options

Product	Cat. #	Size
NanoBRET™ PPI Control Pair (p53/MDM2)	N1641	Each
NanoBRET™ BRD 4/Histone H3.3 Interaction Assay	N1830	Each
NanoBRET™ cMyc/MAX Interaction Assay	N1870	Each

Tailored R&D Solutions offers additional predesigned NanoBRET PPI assays, including interactions for:

- Ras/Raf signaling
- Kinases
- Membrane receptors
- And more!

Tailored R&D Solutions can also build custom PPI assay vectors for proteins of your choice.

Visit **www.promega.com/custom-solutions/tailored-solutions** or **TailoredSolutions@promega.com** to learn more.

Recent Citations:

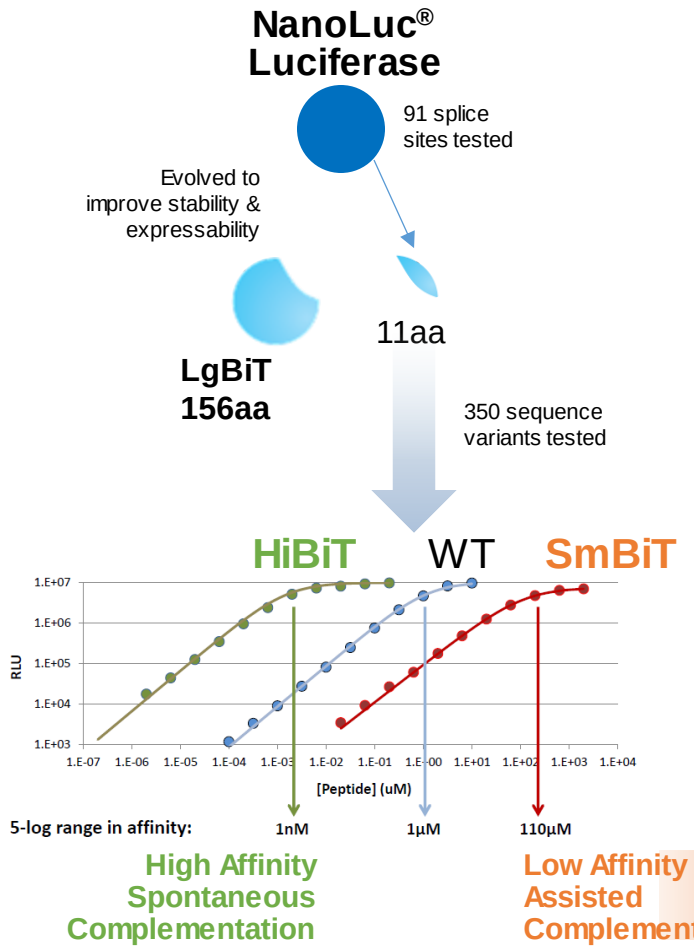
Olp, M.D., *et al.* (2020) Covalent-Fragment Screening of BRD4 Identifies a Ligandable Site Orthogonal to the Acetyl-Lysine Binding Sites. *ACS Chem. Biol.* **15**, 1036-1049.

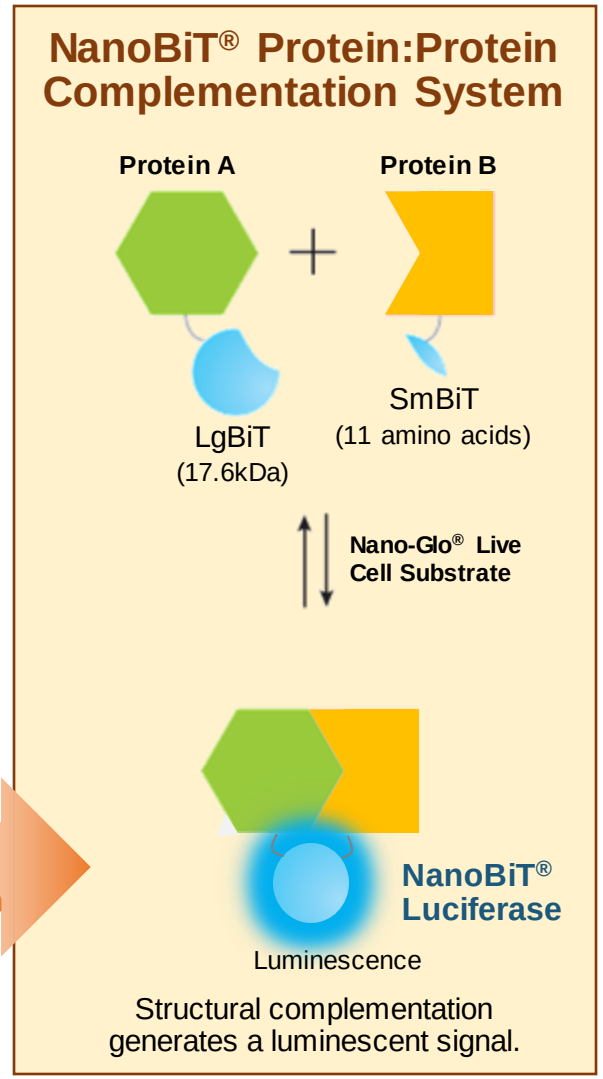
Li, K., *et al.* (2020) TRIB3 promotes MYC-associated lymphoma development through suppression of UBE3B-mediated MYC degradation. *Nat. Comm.* **11**, 6316.

Promega

NanoBiT® Structural Complementation Technology

Low-affinity, assisted complementation subunits SmBiT and LgBiT





Read about the design and use of this assay.

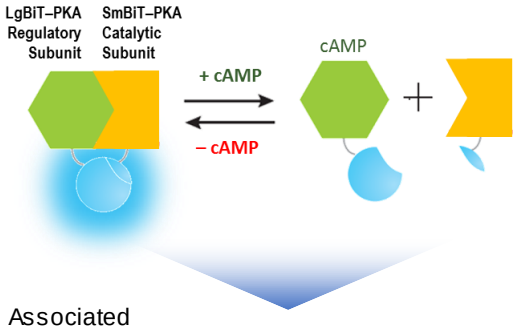
Dixon, A.S., *et al.* (2016) NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem. Biol.* 11, 400-8.

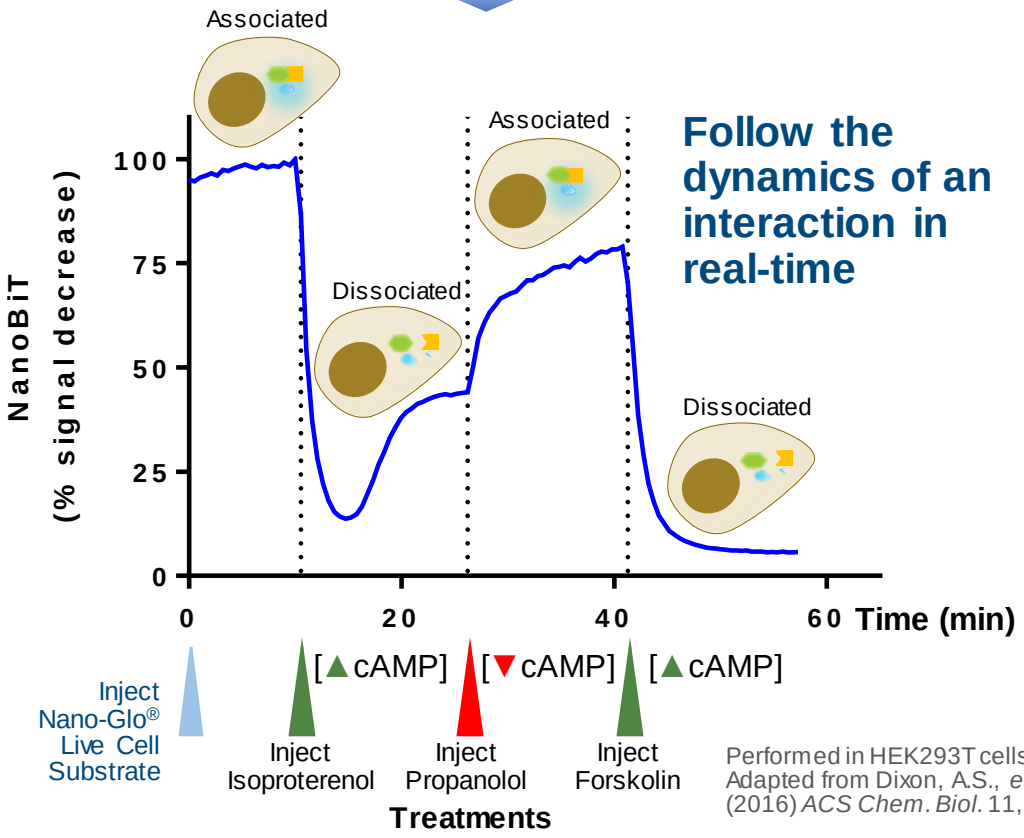
NanoBiT® Assays for Protein Interactions

Exciting possibilities for exploring protein interactions in live cells

NanoBiT™ Positive Control:

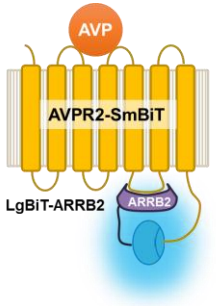
cAMP control of Protein Kinase A (PKA) regulatory & catalytic subunit interaction



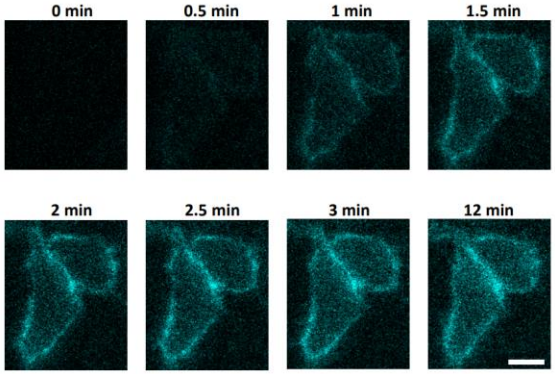


Follow the dynamics of an interaction in real-time

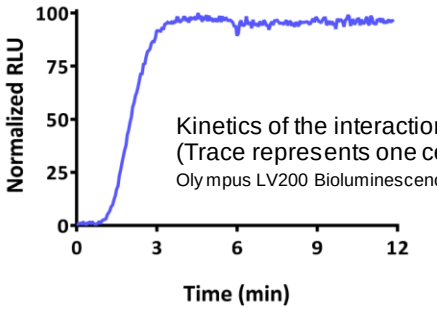
Performed in HEK293T cells at 28°C. Adapted from Dixon, A.S., *et al.* (2016) *ACS Chem. Biol.* 11, 400-8.



Interaction of Arginine Vasopressin Receptor and β-arrestin in the presence of arginine vasopressin.



Bioluminescent imaging of the interaction in HeLa cells following arginine vasopression (1µM) addition.
Olympus LV200 Bioluminescence Imaging System



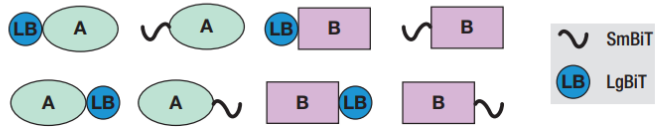
Adapted from Dixon, A.S., *et al.* (2016) *ACS Chem. Biol.* **11**, 400-8.

Promega

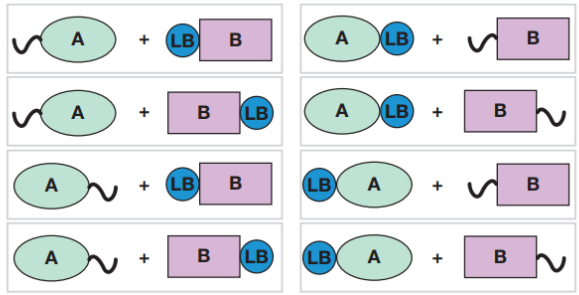
NanoBiT® PPI Starter Systems
SmBiT and LgBiT Fusion Vector Sets & Detection Reagents

Generating and optimizing a NanoBiT® assay

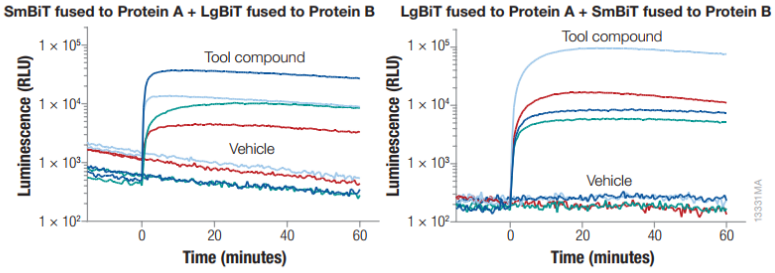
1. Generate constructs encoding LgBiT and SmBiT fusions to protein A and protein B (up to 8 possible constructs).



2. Transiently transfect the different plasmid combinations into cells (up to 8 possible combinations).

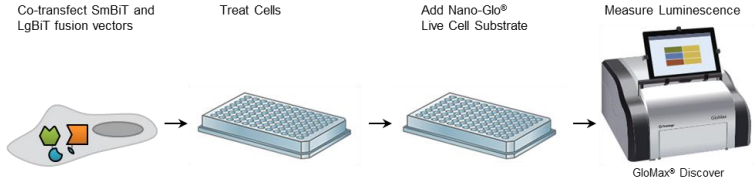


3. Screen for an optimal orientation by comparing tool compound treatment to vehicle treatment.



4. Select an orientation showing maximal fold response.

NanoBiT® Assay Workflow



The **NanoBiT® PPI Starter Systems** provide the vectors required to create LgBiT and SmBiT protein fusions, along with control vectors and the **Nano-Glo® Live Cell Assay System**, a single-addition, non-lytic detection reagent used for monitoring NanoBiT® luminescence in living cells. See below for details of the vectors provided with each system. Live-cell reagents allow you to detect protein:protein interactions in real time using full-length proteins or fragments.

- Detects interactions at low expression levels and allows real-time kinetic analysis in live cells
- Reversible protein complementation reporter, allows analysis of protein association and disassociation
- Improved sensitivity and accuracy over split firefly luciferase

Product	Cat. #	Size
NanoBiT® PPI MCS Starter System	N2014	Each
NanoBiT® PPI Flexi® Starter System	N2015	Each
Nano-Glo® Live Cell Assay	N2011	100 assays
	N2012	1,000 assays
	N2013	10,000 assays

Tailored R&D Solutions offers predesigned NanoBiT® PPI assays, including interactions for:

- B-Arrestin recruitment
- Nuclear receptors
- And more!

Tailored R&D Solutions can also build custom PPI vectors for proteins of your choice.

Visit **www.promega.com/custom-solutions/tailored-solutions** or **TailoredSolutions@promega.com** to learn more.

Recent Citations:

Read, M.L., *et al.* (2022) Targeting non-canonical pathways as a strategy to modulate the sodium iodide symporter. *Cell Chem. Biol.* **29**, 502-516.e7.

Janssens, L.K. and Stove, C.P. (2021) Sensing an Oxygen Sensor: Development and Application of Activity-Based Assays Directly Monitoring HIF Heterodimerization. *Anal. Chem.* **93**, 14462-14470.

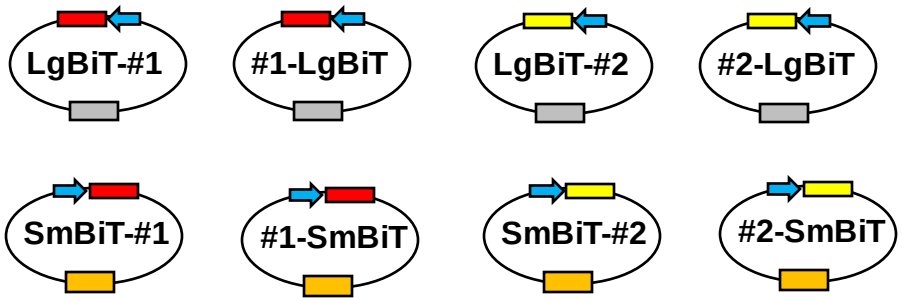
Meyrath, M., *et al.* (2020) The atypical chemokine receptor ACKR3/CXCR7 is a broad spectrum scavenger for opioid peptides. *Nat. Comm.* **11**, 3033.

NanoBiT® BiBiT Ready Vectors

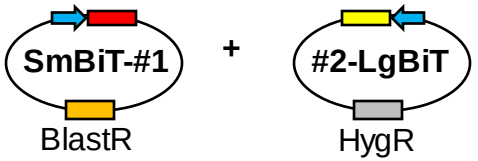
Express SmBiT and LgBiT Fusions From One Bidirectional Vector

Generating and optimizing a BiBiT vector

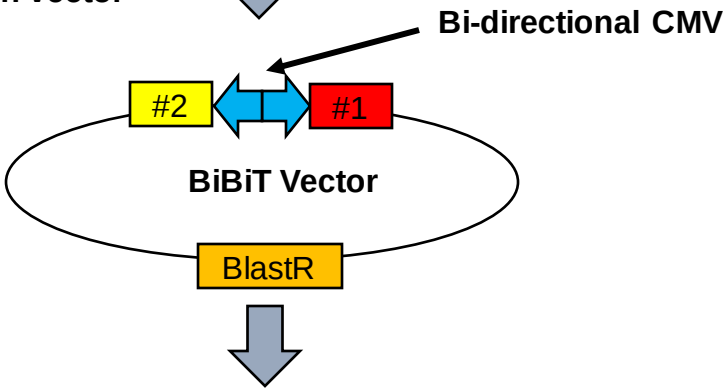
1. Make up to 8X different constructs



2. Screen for optimal orientation



3. Ligate fragments from each vector



4. Create stable cell lines via random integration

The **NanoBiT® BiBiT Ready Vectors** provide a simpler way to generate stable cells lines for NanoBiT® PPI assays. A single, bidirectional vector expresses both the LgBiT and SmBiT protein fusions, eliminating the need to co-transfect multiple plasmids and reducing assay variability.

Product	Cat. #	Size
NanoBiT® MCS BiBiT Ready Vectors	CS1603B32	Each
NanoBiT® Flexi® BiBiT Ready Vectors	CS1603B33	Each

The BiBiT Ready Vectors are Early Access Materials. Please contact your local Promega Representative or TailoredSolutions@promega.com to order.

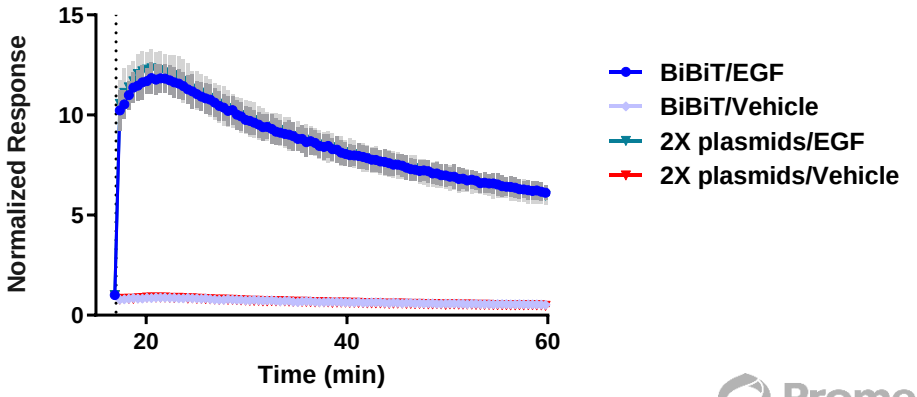
Tailored R&D Solutions offers predesigned BiBiT PPI assays, including interactions for:

- Ras/Raf signaling
- Membrane receptors
- And more!

Tailored R&D Solutions can also build custom BiBiT vectors for proteins of your choice.

Visit **www.promega.com/custom-solutions/tailored-solutions** or **TailoredSolutions@promega.com** to learn more.

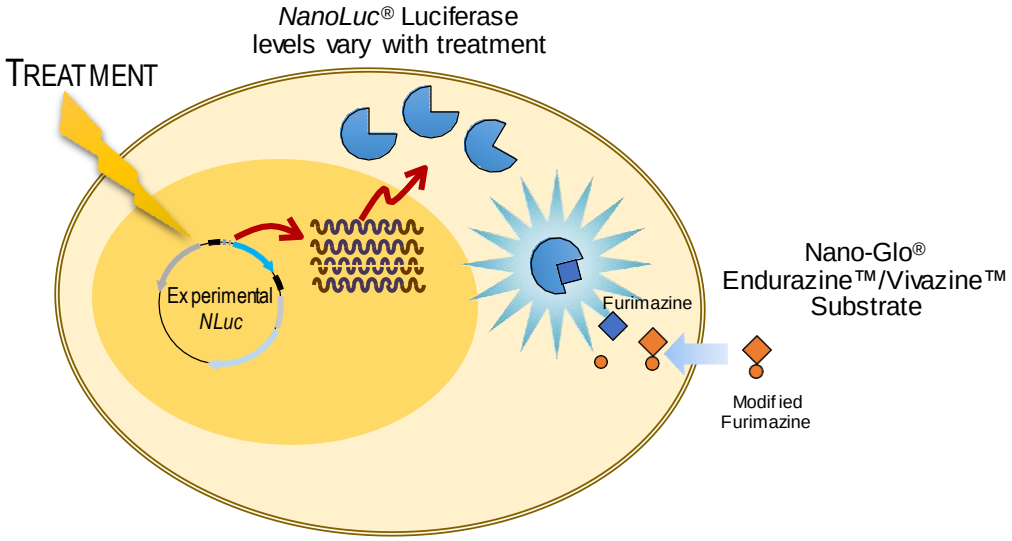
BiBiT vs 2X plasmids - EGFR



Promega

Nano-Glo® Extended Live Cell Substrates

Measures NanoLuc® Luminescence in Living Cells for Hours or Days

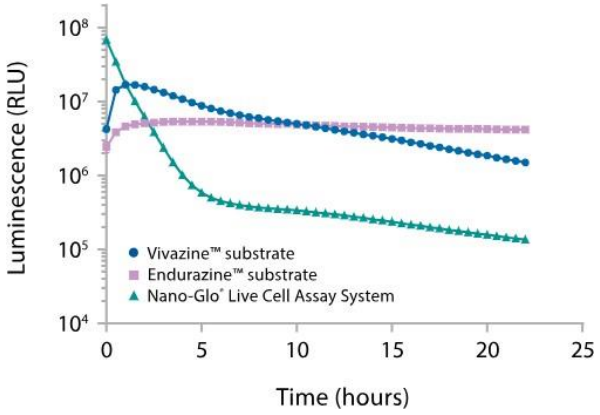


The Nano-Glo® Endurazine™ and Vivazine™ Extended Live Cell Substrates provide alternative live-cell-detection methods for NanoLuc® and NanoBiT® luciferases that enable nonlytic assays for periods lasting several hours to days. For both substrates, a slow rate of ester hydrolysis leads to the steady release of furimazine throughout the experiment, a process catalyzed by cellular esterases. Once formed, furimazine serves as a substrate for NanoLuc® and NanoBiT® Luciferases. The Vivazine™ Substrate typically shows increased brightness but also an increased rate of signal decay compared to the Endurazine™ Substrate. The Endurazine™ Substrate will provide the maximum signal stability but lower initial signal intensity compared to the other Nano-Glo® Live Cell Substrates.

- Increased signal stability for extended real-time kinetic analysis of reporter activity
- Simplify time course studies by measuring response in the same sample over time
- Sensitivity to measure protein at endogenous levels—no overexpression required

Product	Cat. #	Size
Nano-Glo® Endurazine™ Live Cell Substrate	N2570	0.1ml
	N2571	1ml
	N2572	10ml
Nano-Glo® Vivazine ™ Live Cell Substrate	N2580	0.1ml
	N2581	1 ml
	N2582	10 ml
Nano-Glo® Extended Live Cell Substrate Trial Pack *(N2570 + N2580)*	N2590	2 x 0.1ml

Choose the Best Combination of Signal Intensity and Stability for Your Experiment



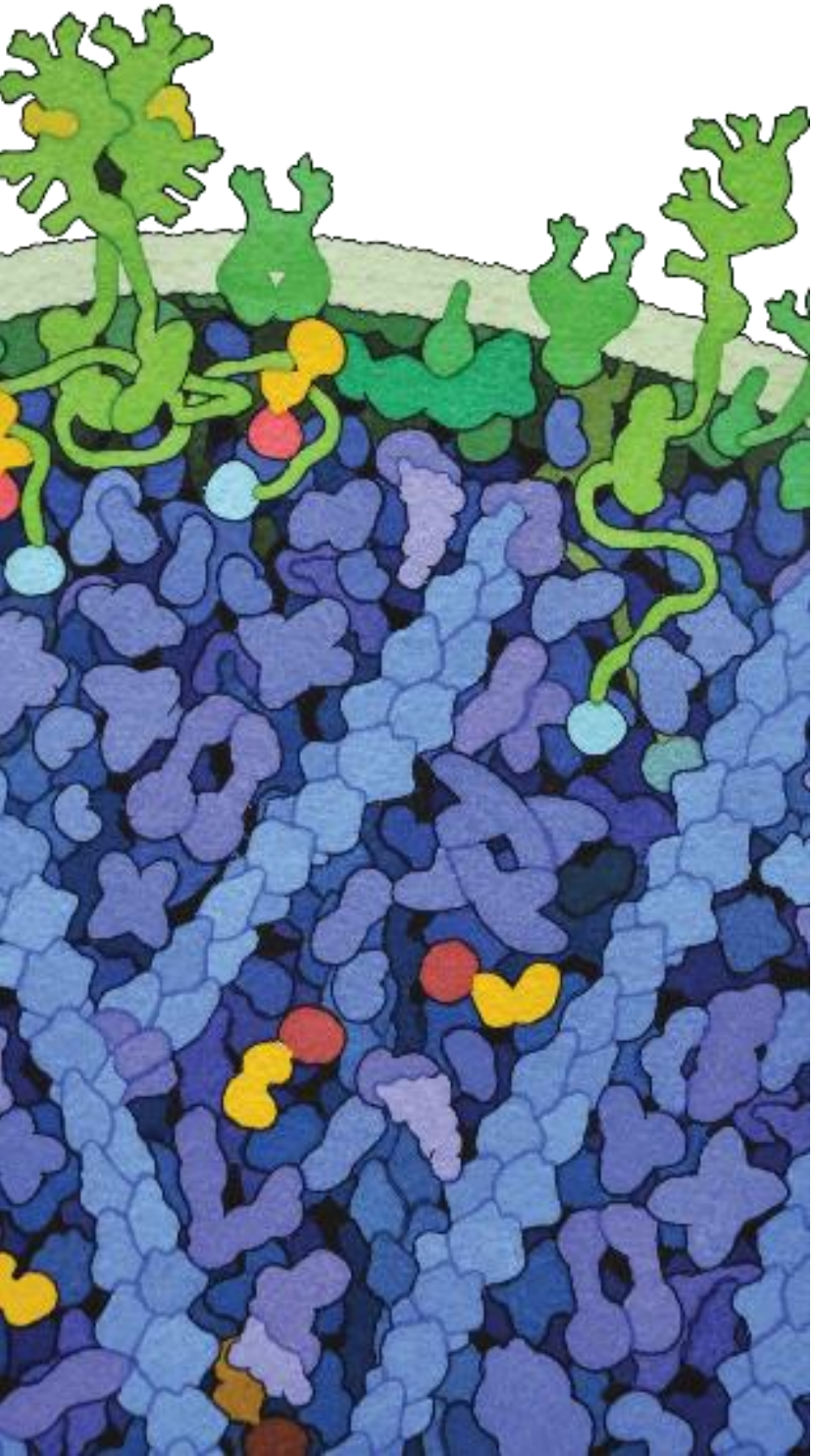
NanoLuc® luciferase expressed from the CMV promoter was transiently transfected into HEK293 cells using 1ng vector DNA/well in a 96-well plate. The average luminescence is plotted (n = 3) with error bars representing the standard deviation.

Recent Citations (Endurazine™ Substrate):

Carrera, S., *et al.* (2021) Complexities in the role of acetylation dynamics in modifying inducible gene activation parameters. *Nucleic Acids Res.* **49**, 12744-12756.

Bauerscachs, H.G., *et al.* (2022) N-methyl-D-aspartate Receptor-mediated Preconditioning Mitigates Excitotoxicity in Human Induced Pluripotent Stem Cell-derived Brain Organoids. *Neurosci.* **484**, 83-97.

Truong, D.J., *et al.* (2022) Intron-encoded cistronic transcripts for minimally invasive monitoring of coding and non-coding RNAs. *Nat. Cell Biol.* **24**, 1666-1676.

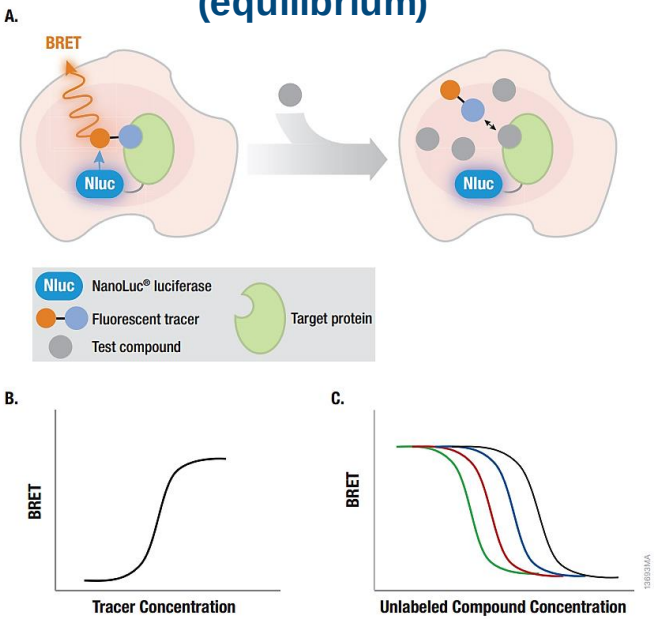


Measuring Protein:Ligand Interactions

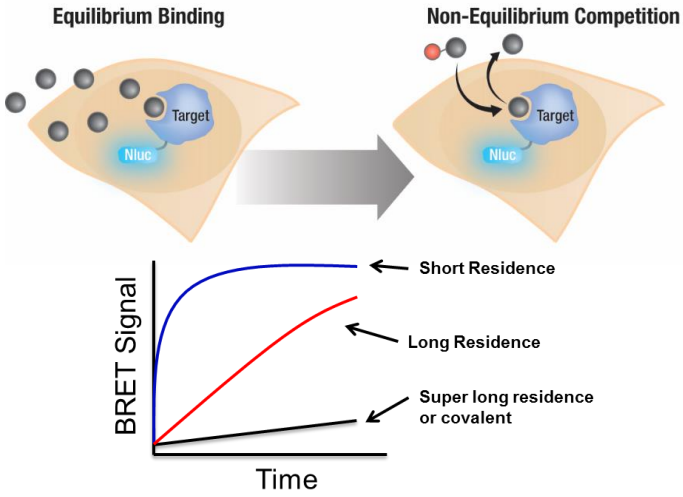
NanoBRET™ Target Engagement Assays

Measure compound binding affinity & residence time in live cells

Test Compound Affinity (equilibrium)



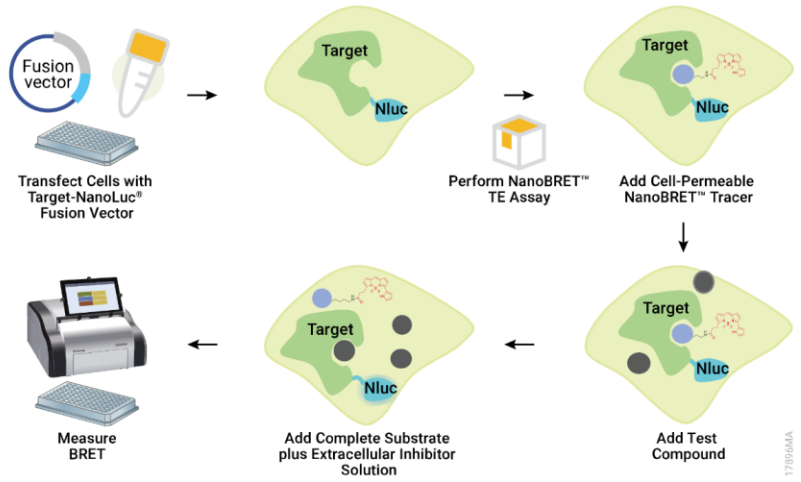
Test Compound Residence Time (non-equilibrium)



Key Features

- **Measure Target Engagement in Live Cells:** Measure test compound affinity and occupancy in live cells for more physiologically relevant information.
- **Directly Measure Residence Time:** Compound and tracer compete directly for the binding site.
- **Use Full-Length Protein:** Assay measures interaction with full-length proteins that are similar to the native forms.
- **Express Target Proteins at Low Levels:** The brightness of NanoLuc luciferase allows for very low expression levels, which may be comparable to endogenous levels

Assay workflow



[NanoBRET™ TE Assay Overview]

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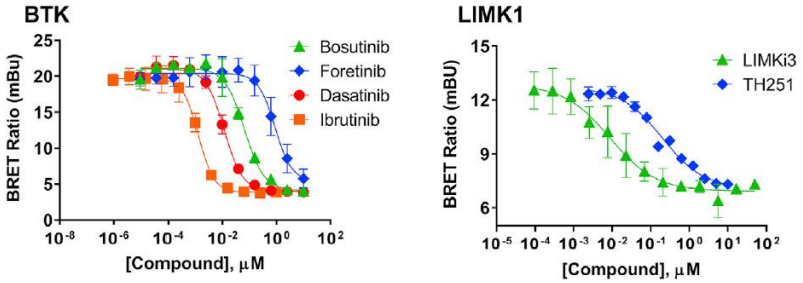
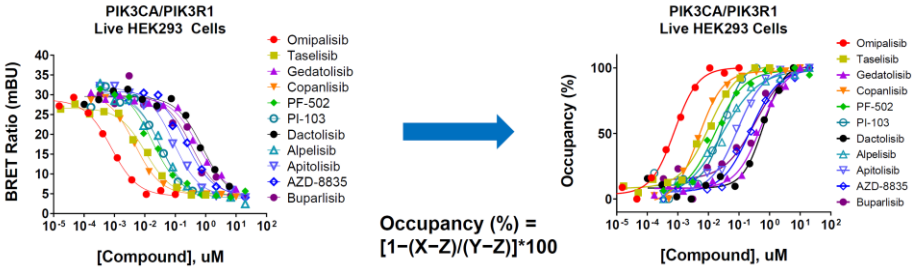
NanoBRET™ TE Intracellular Kinase Assays

Measure Compound Affinity and Occupancy at Kinase Targets in Intact Cells

Measure affinity profiles of various inhibitor classes that disrupt binding of the probe to the active site.



NanoBRET™ TE Kinase Assays determine the fractional occupancy or the amount of compound bound to a kinase



- Controls for background (Z) & max BRET (Y) allow quantitative compound fractional occupancy determination from compound BRET value (X)

Read the paper about the design and use of this assay

Vasta, J.D., *et al.* (2018) Quantitative, wide-spectrum kinase profiling in live cells for assessing the effect of cellular ATP on target engagement. *Cell Chem. Biol.* **25**, 1-9.

Using the **NanoBRET™ TE Kinase Assay**, a fixed concentration of tracer is added to cells expressing the desired kinase-NanoLuc® fusion to generate a BRET signal. Introduction of competing compounds results in a dose-dependent decrease in NanoBRET™ signal, which allows quantitation of the intracellular affinity of the target protein for the test compound.

- Directly measure kinase-compound affinity and occupancy
- Enable assays for >340 kinases
- Multi-well plate format that yields high quality data, suitable for screening inhibitors

Product	Cat. #	Size*
NanoBRET™ TE Intracellular Kinase Assay, K-3	N2600	100 assays
NanoBRET™ TE Intracellular Kinase Assay, K-4	N2520	100 assays
NanoBRET™ TE Intracellular Kinase Assay, K-5	N2500	100 assays
NanoBRET™ TE Intracellular Kinase Assay, K-8	N2620	100 assays
NanoBRET™ TE Intracellular Kinase Assay, K-9	N2630	100 assays
NanoBRET™ TE Intracellular Kinase Assay, K-10	N2640	100 assays
NanoBRET™ TE Intracellular Kinase Assay, K-11	N2650	100 assays
NanoBRET™ TE Intracellular Kinase Assay, K-12	NF1001	1,000 assays

Accessory Products

Product	Cat. #	Size
CC1 pan-Kinase Inhibitor	N2661	100µl
TransfectNow™ HEK293 Cells	NC1001	1 x 0.5ml
	NC1002	2 x 1ml

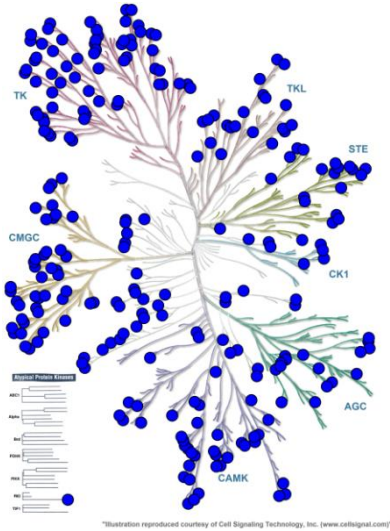
Recent Citations:

Jin, H.Y., *et al.* (2020) High-Throughput Implementation of the NanoBRET Target Engagement Intracellular Kinase Assay to Reveal Differential Compound Engagement by SIK2/3 Isoforms. *SLAS Discov.* **25**, 215-222.

Gallo, D., *et al.* (2022) CCNE1 amplification is synthetic lethal with PKMYT1 kinase inhibition. *Nature*. **604**, 749-756.

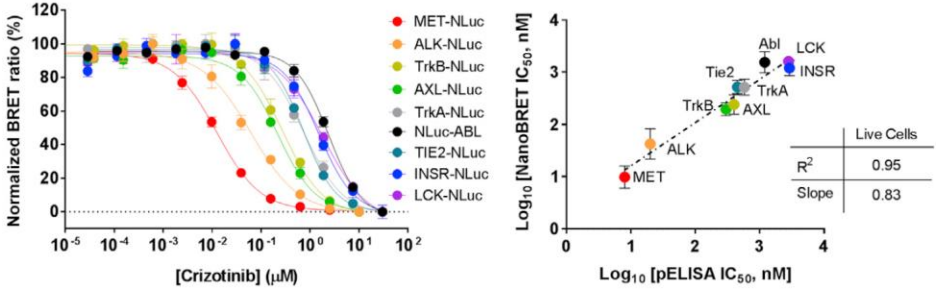
NanoLuc®-Kinase Fusions for use with the NanoBRET™ TE Intracellular Kinase Assay

>340 live cell kinases spanning the kinome, including full-length wild-type and mutant kinases. Learn more here.



"Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)"

NanoBRET™ TE Kinase Assay results correlate well with published phospho-ELISA data



Kinase-NanoLuc® Fusion Vectors for NanoBRET™ Target Engagement

- Transfection-ready DNA to express full-length kinase fused to NanoLuc® luciferase at either the N-terminus or C-terminus
- Use with NanoBRET™ TE Intracellular Kinase Assays to measure kinase target engagement in live cells
- Each kinase fusion supplied with recommended tracer and tracer concentration for intracellular target engagement work.
- A full list with Application Notes at Kinase Target Engagement Assay Selection Table

Application Notes
demonstrating tracer binding affinity in live cells and providing recommended tracer concentration for each Kinase-NanoLuc® Fusion, allowing quick start-up of the NanoBRET™ TE Intracellular Kinase Assays

NanoBRET™ TE Intracellular Kinase Assays

- Pair recommended NanoBRET™ TE Kinase Assay with Kinase-NanoLuc® fusion vector.
- NanoBRET™ TE Kinase Assays come with the following reagents to perform the assay, including cell-permeable tracer, tracer dilution buffer, Intracellular TE Nano-Glo® substrate/inhibitor and Extracellular NanoLuc® Inhibitor.
- See a full list of NanoBRET™ TE Kinase Assays on the product page.

Tailored R&D Solutions can also build custom kinase TE assays and offer compound profiling services against kinases using the NanoBRET™ TE assays

Visit **www.promega.com/custom-solutions/tailored-solutions** or email **TailoredSolutions@promega.com** to learn more.

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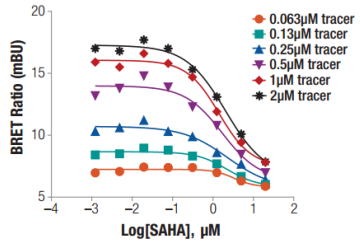
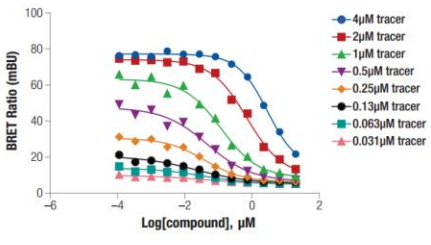
NanoBRET™ TE Intracellular HDAC and BET BRD Assay

Directly Measure HDAC/BET BRD-Test Compound Affinity & Residence Time in Live Cells

Inhibition of NanoBRET ™ Tracer binding to transiently expressed NLuc-HDAC6 by SAHA (left) and NLuc-BRD4 by iBET151 (right) in HEK293 cells.

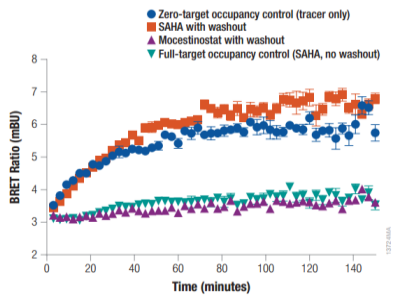


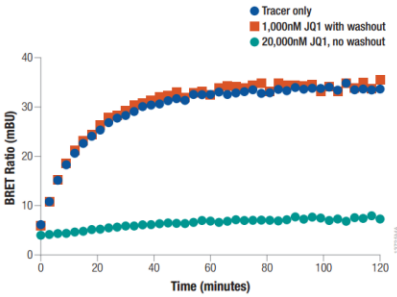
Tracer Concentration (µM)	2	1	0.5	0.25	0.13	0.063
IC_{50}	2.07	**1.42**	1.71	2.27	2.23	3.32



Tracer Concentration (µM)	4	2	1	0.5	0.25	0.13	0.063	0.031
IC_{50}	2.9	0.71	0.12	**0.048**	0.034	0.022	0.040	0.043

Drug residency time measurement for HDAC1-Nluc (left) and NLuc-BRD4 (right) transiently expressed in HeLa Cells





Read the paper about the design and use of this assay

Robers, M.B., *et al*. (2015) Target engagement and drug residence time can be observed in living cells w ith BRET. *Nature Comm*. **6**, 10091.

The NanoBRET™ Target Engagement (TE) Assay measures compound binding at select target proteins in intact cells, in real time. The NanoBRET™ TE Assay uses four key components: An expressed cellular target protein that is fused to the bright NanoLuc® luciferase; a cell-permeable fluorescent tracer that specifically binds to the target protein; a substrate for NanoLuc® luciferase; and a cell-impermeable inhibitor for NanoLuc® luciferase.

- Compound and tracer compete directly for the binding site
- Assays rely on full-length proteins that are similar to the native forms
- Express target proteins at low levels

Product	Cat. #	Size
NanoBRET™ TE Intracellular HDAC Assay	N2080	100 assays
	N2081	1,000 assays
NanoBRET™ TE Intracellular HDAC Assay + DNA Bundle	N2170	1,000 assays
NanoBRET™ TE Intracellular HDAC Assay Reagent Refill	N2090	10,000 assays
NanoBRET™ TE Intracellular BET BRD Assay	N2130	100 assays
	N2131	1,000 assays
NanoBRET™ TE Intracellular BET BRD Assay + DNA Bundle	N2180	1,000 assays
NanoBRET™ TE Intracellular BET BRD Assay Reagent Refill	N2140	10,000 assays

Recent Citations:

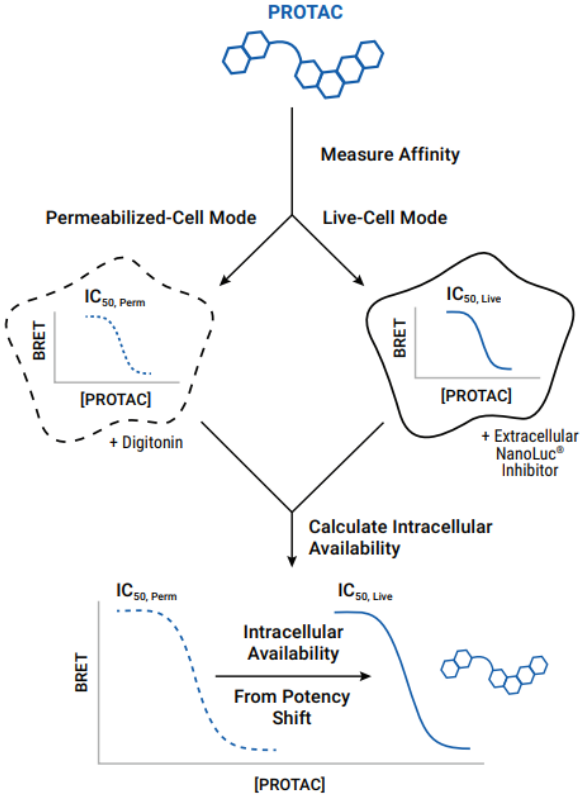
Asawa, R.R., *et al.* (2020) A Comparative Study of Target Engagement Assays for HDAC1 Inhibitor Profiling. *SLAS Discov.* **25**, 253-264.

Modukuri, R.K., *et al*. (2022) Discovery of potent BET bromodomain 1 stereoselective inhibitors using DNA-encoded chemical library selections. *PNAS*. **119**, e2122506119.

NanoBRET™ TE Intracellular E3 Ligase Assays

Measure E3 Ligase Target Engagement and Compound Intracellular Availability

Workflow for evaluating target binding and intracellular availability

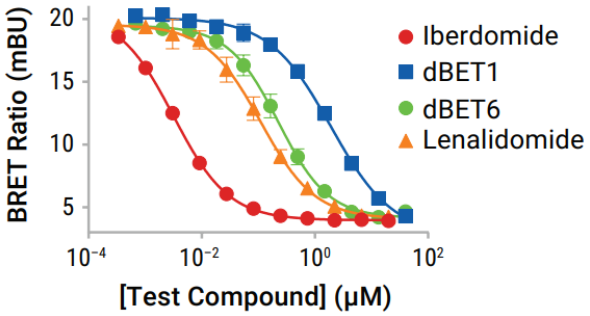


$$\text{Relative Binding Affinity (RBA}_{\text{compound}}) = \frac{IC_{50,\ \text{Live}}}{IC_{50,\ \text{permeabilized}}}$$

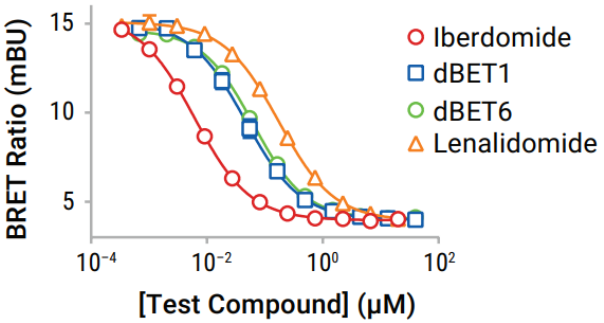
$$\text{Availability Index (AI)} = \frac{RBA_{\text{PROTAC}}}{RBA_{\text{control.}}}$$

- AI = 1 for control
- AI >1 : less available than control
- AI <1 : more available than control

Compound-CRBN binding in live-cell mode



Compound-CRBN binding in permeabilized-cell mode



CRBN Ligand Affinity and Availability Index Parameters

CRBN Ligand Affinity and Availability Index Parameters

Compound	Live-Cell Affinity (nM)	Permeabilized-Cell Affinity (nM)	RBA	AI
Lenalidomide*	110 ± 8	170 ± 8	0.63 ± 0.06	1.0 ± 0.1
Iberdomide	3.2 ± 0.2	6.3 ± 0.3	0.50 ± 0.04	0.79 ± 0.09
dBET1	1900 ± 150	50 ± 3	38 ± 4	60 ± 8
dBET6	220 ± 10	63 ± 2	3.5 ± 0.2	5.6 ± 0.6

*Lenalidomide served as the permeable control compound for this analysis.

Protein degradation via small molecules such as PROTACs or molecular glues is governed by multiple steps. The initial steps involve entry of the small molecule degrader compound into the cell, follow ed by binding to the E3 ubiquitin ligase and protein target. The NanoBRET™ TE Intracellular E3 Ligase Assays quantitate both intracellular target engagement and intracellular degrader compound availability.

- Quantitate intracellular affinity of compounds for CRBN or VHL E3 ubiquitin ligases in live cells
- Simple w orkflow for calculating compound intracellular availability, a measure of permeability
- Scalable assays using addition-only protocols

Product	Cat. #	Size
NanoBRET™ TE Intracellular E3 Ligase Assay, CRBN	N2910	100 assays
	N2911	1,000 assays
NanoBRET™ TE Intracellular E3 Ligase Detection Reagents, CRBN	N2912	10,000 assays
NanoBRET™ TE Intracellular E3 Ligase Assay, VHL	N2930	100 assays
	N2931	1,000 assays
NanoBRET™ TE Intracellular E3 Ligase Detection Reagents, VHL	N2932	10,000 assays

Recent Citations:

Guo, W.H., *et al.* (2020) Enhancing intracellular accumulation and target engagement of PROTACs w ith reversible covalent chemistry. *Nat. Comm.* **11**, 4268.

Kurimchak, A.M., *et al.* (2022) The drug efflux pump MDR1 promotes intrinsic and acquired resistance to PROTACs in cancer cells. *Sci. Signal.* **15**, eabn2707.

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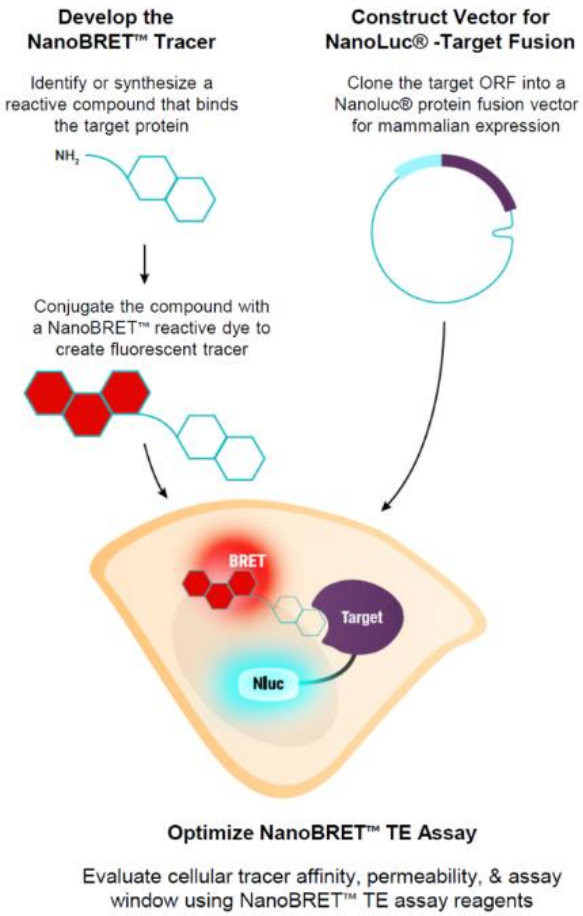
NanoBRET™ TE Assays for Other Targets

Beyond the target proteins available in catalog

Design your own Target Engagement assay



Tools for developing NanoBRET™ TE Assay

NanoBRET™ Dyes	Cat. #	Size
NanoBRET™ 618 TFP (Tetrafluoryl Phenyl)	CS189405	5mg
NanoBRET™ 590 SE (Succinimydal Ester)	CS189406	5mg
	CS189407	100mg
NanoBRET™ 590 Azide-C3	CS189408	5mg
NanoBRET™ 590 PEG(O4) SE	CS189409	5mg
NanoBRET™ 590 C4 SE	CS189410	5mg
NanoBRET™ 590 Carboxylic Acid	CS189411	5mg
NanoBRET™ 590 Maleimide C2	CS189412	5mg

NanoLuc® Cloning Vectors	Cat. #	Size
pNLF1-N [CMV/Hygro] Vector	N1351	20µg
pNLF1-C [CMV/Hygro] Vector	N1361	20µg
pFN31A Nluc CMV-Hygro Flexi® Vector	N1311	20µg
pFN31K Nluc CMV-neo Flexi® Vector	N1321	20µg
pFC32A Nluc CMV-Hygro Flexi® Vector	N1331	20µg
pFC32K Nluc CMV-neo Flexi® Vector	N1341	20µg

NanoBRET™ TE Assay Reagents	Cat. #	Size
Intracellular TE Nano-Glo® Substrate/Inhibitor	N2160	1,000 assays
	N2161	10,000 assays
	N2162	100 assays
Tracer Dilution Buffer	N2191	50ml
Transfection Carrier DNA	E4881	5 x 20µg
	E4882	2 x 100µg

Validated NanoBRET™ TE Assays in Early Access

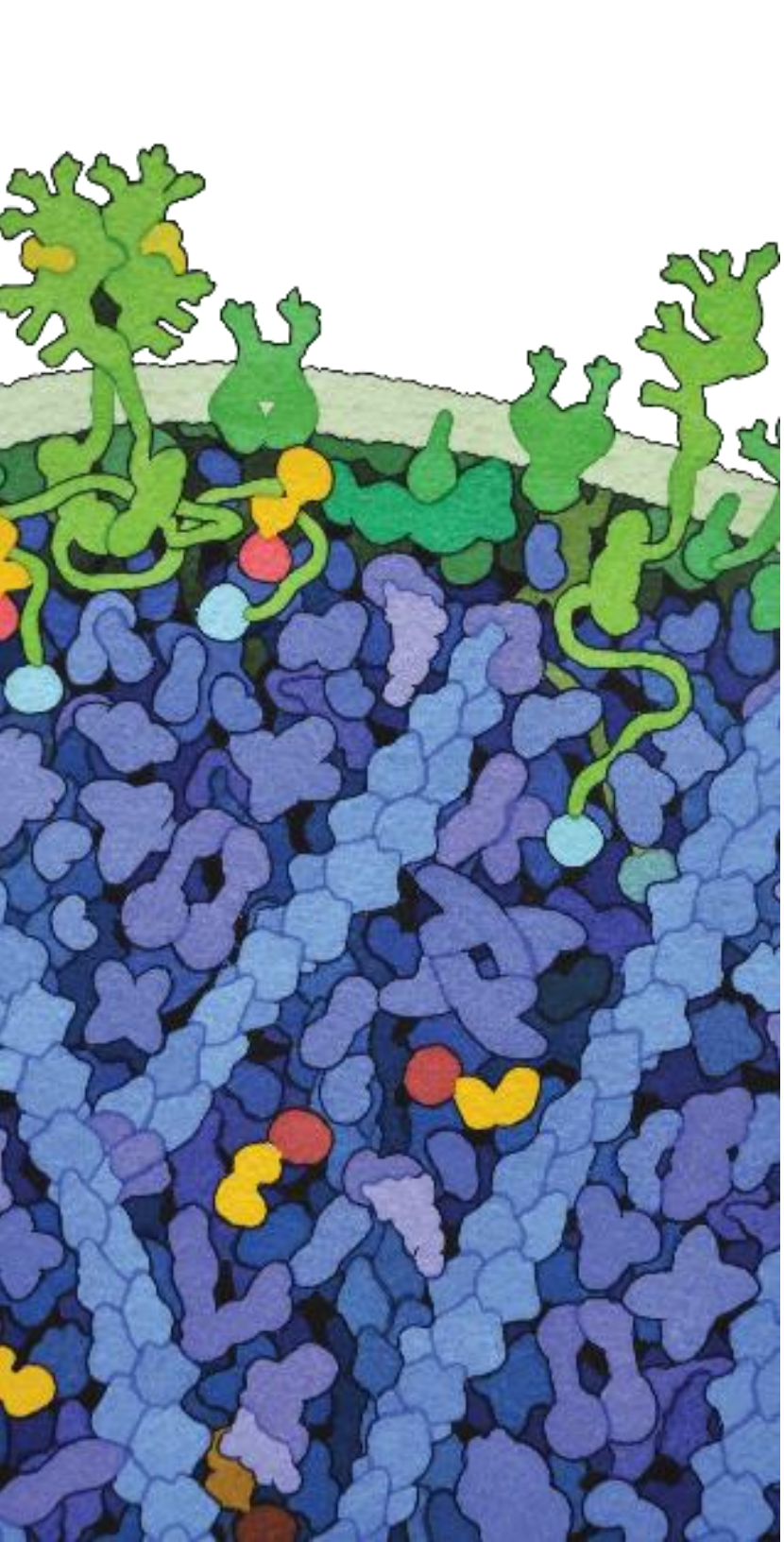
Tailored R&D Solutions offers additional NanoBRET™ Target Engagement assays, including targets in the following protein family

- E3 Ubiquitin Ligase Complex
- Bromodomains
- G-protein Coupled Receptors
- Heat Shock Proteins
- Kinases
- NLR
- PARP
- RAF/RAS

Contact your local Promega representative or email the Tailored R&D Solutions team at **TailoredSolutions@promega.com** to learn more.

Read the paper about designing NanoBRET™ TE Assays

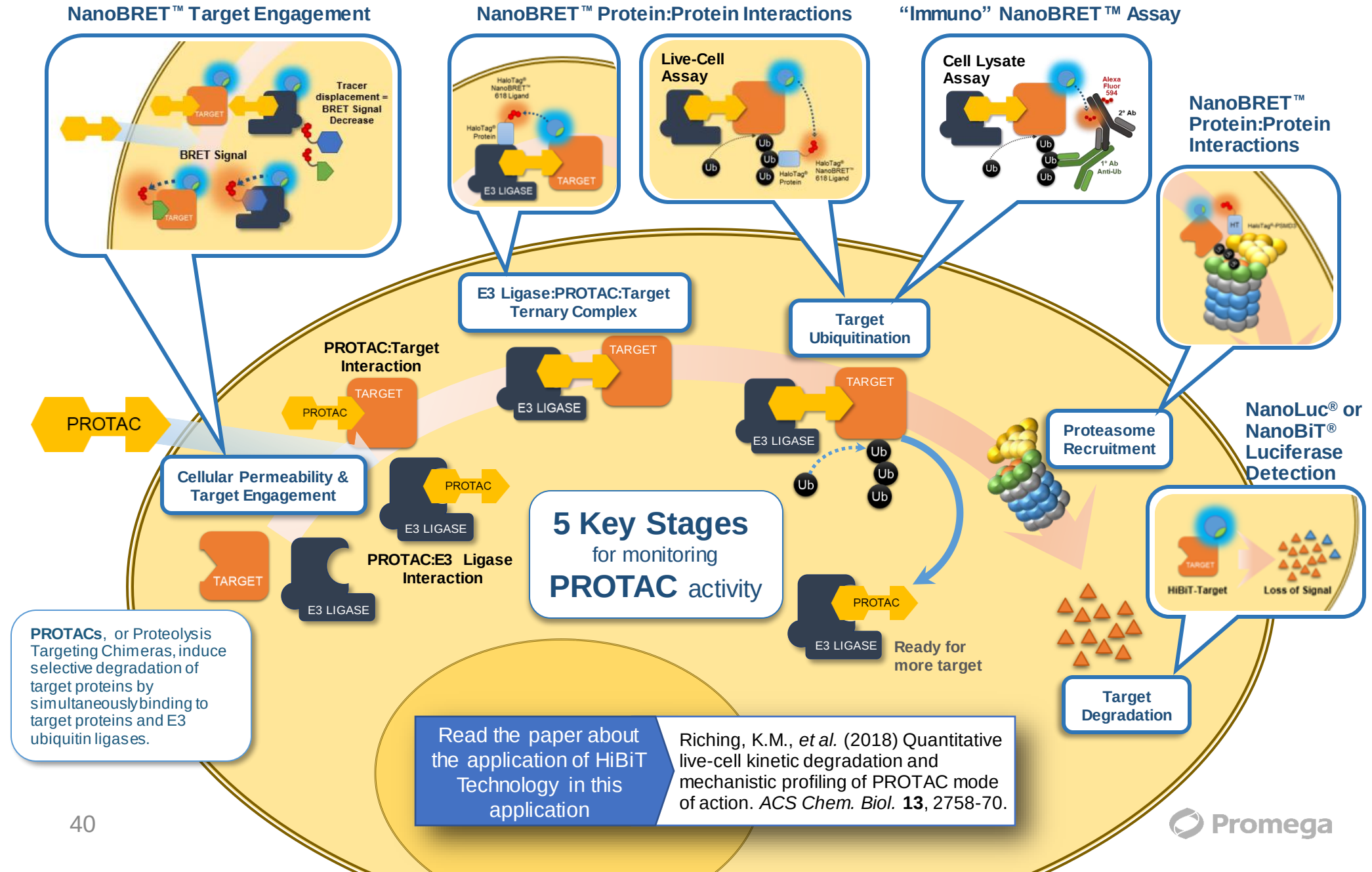
Robers, M.B., *et al*. (2019) Quantitative, Real-Time Measurements of Intracellular Target Engagement Using Energy Transfer. *Methods Mol. Biol*. **1888**, 45-71.



NanoLuc® Protein Reporter Application Examples

Monitoring PROTAC-directed Target Degradation

Learn more about tools for this application



Read the paper about the application of HiBiT Technology in this application

Riching, K.M., *et al.* (2018) Quantitative live-cell kinetic degradation and mechanistic profiling of PROTAC mode of action. *ACS Chem. Biol.* **13**, 2758-70.

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GPCR Analysis
Application of NanoLuc® Protein Reporter Technologies

Pre-cloned β-Arrestin 1 & 2 with either SmBiT or LgBiT at the N- or C-terminus available through Tailored R&D Solutions TRSOrder Support@promega.com

NanoBRET™ PPI Assay
Schihada, H., *et al.* (2018) A universal bioluminescence resonance energy transfer sensor design enables high-sensitivity screening of GPCR activation dynamics. *Comm. Biol.* **1**, 105

NanoBRET™ Target Engagement Strategy
Conroy, S., *et al.* (2018) Synthesis and evaluation of the first fluorescent antagonists of the human P2Y2 receptor based on AR-C118925. *J. Med. Chem.* **61**, 3089-113.

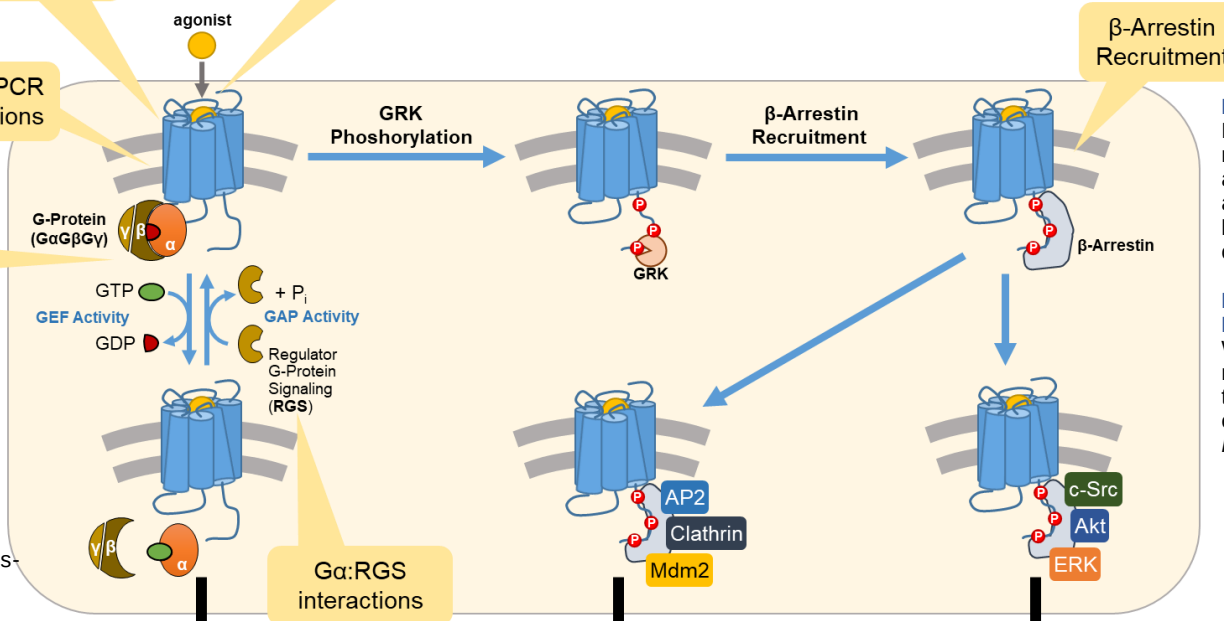
Stoddart, L.A., *et al.* (2018) Development of novel fluorescent histamine H1-receptor antagonists to study ligand-binding kinetics in living cells. *Sci. Reports* **8**, 1572.

NanoBiT® PPI Assay
He, S.-Q., *et al.* (2018) Oligomerization of MrgC11 and μ-opioid receptors in sensory neurons enhances morphine analgesia. *Sci. Signal.* **11**, eaao3134.

Qian, M., *et al.* (2018) Design, synthesis, and biological evaluation of bivalent ligands targeting dopamine D2-like receptors and the μ-opioid receptor. *ChemMedChem* **13**, 944-56.

Storme, J., *et al.* (2018) Molecular dissection of the human A3 adenosine receptor coupling w ith β-arrestin2. *Biochem. Pharmacol.* **148**, 298-307.

NanoLuc® Luciferase Donor/FP Acceptor
White, C.W., *et al.* (2017) Using nanoBRET and CRISPR/Cas9 to monitor proximity to a genome edited protein in real-time. *Sci. Reports* **7**, 3187.



NanoBRET™ PPI Assay
Lu, J., *et al.* (2017) Dopamine D2 receptor and β-arrestin 2 mediate amyloid-β elevation induced by anti-Parkinson's disease drugs, levodopa and piribedil, in neuronal cells. *PLoS One* **12**, e0173240.

NanoLuc® Luciferase Donor/FP Acceptor
White, C.W., *et al.* (2017) Using nanoBRET and CRISPR/Cas9 to monitor proximity to a genome edited protein in real-time. *Sci. Reports* **7**, 3187.

NanoBiT® PPI Assay
Wan, Q., *et al.* (2018) Mini G protein probes for active G protein-coupled receptors (GPCRs) in live cells. *J. Biol. Chem.* **293**, 7466-73.

GloSensor™ cAMP Assay
Nagata, T., *et al.* (2018) An all-trans-retinal-binding opsin peropsin as a potential dark-active and light-inactivated G protein-coupled receptor. *Sci. Reports* **8**, 3535.
A firefly luciferase-based biosensor.

Intracellular Ca^{2+} NanoLuc® BRET Sensors:
Qian, Y., et al. (2018) A bioluminescent Ca^{2+} indicator based on a topological variant of GCaMP6s. *ChemBiochem* DOI: 10.1002/cbic.201800255

NanoBiT® PPI Assay
Bodle, C.R., *et al.* (2017) Development of a bimolecular luminescence complementation assay for RGS:G protein interaction in cells. *Anal. Biochem.* **522**, 10-17.

HiBiT Protein Tagging System
Reyes-Alcaraz, A., *et al.* (2018) Conformational signatures in β-arrestin2 reveal natural biased agonism at a G-protein coupled receptor. *Comm. Biol.* **1**, 128.

Live-Cell Assays

Virology Applications
Application of NanoLuc® Protein Reporter Technologies

NanoLuc® Luciferase Recombinant Viruses

– ssRNA virus (e.g., Influenza A)

Tran, V., *et al*. (2013) Highly sensitive real-time in vivo imaging of an influenza reporter virus reveals dynamics of replication and spread. *J. Virol*. **87**, 13321-9.

+ ssRNA virus (e.g., Zika Virus)

Mutso, M., *et al*. (2017) Reverse genetic system, genetically stable reporter viruses and packaged subgenomic replicon based on a Brazilian Zika virus isolate. *J. Gen. Virol*. **98**, 2712-24.

dsRNA virus (e.g., Rotavirus)

Kanai, Y., *et al*. (2017) Entirely plasmid-based reverse genetics system for rotaviruses. *PNAS* **114**, 2349-54.

RNA Reverse Transcribed virus (e.g., HIV-1)

Astronomo, R.D., *et al*. (2017) Neutralization takes precedence over IgG or IgA isotype-related functions in mucosal HIV-1 antibody-mediated protection. *EbioMedicine* **14**, 97-111.

DNA Reverse Transcribed virus (e.g., Hepatitis B virus)

Nishitsuji, H., *et al*. (2018) TIP60 complex inhibits HBV transcription. *J. Virol*. **92**, e01788-17.

dsDNA virus (e.g., JC Polyomavirus)

Geoghegan, E.M., *et al*. (2017) Infectious entry and neutralization of pathogenic JC polyomaviruses. *Cell Rep*. **21**, 1169-79

HiBiT-Tagged Recombinant Viruses

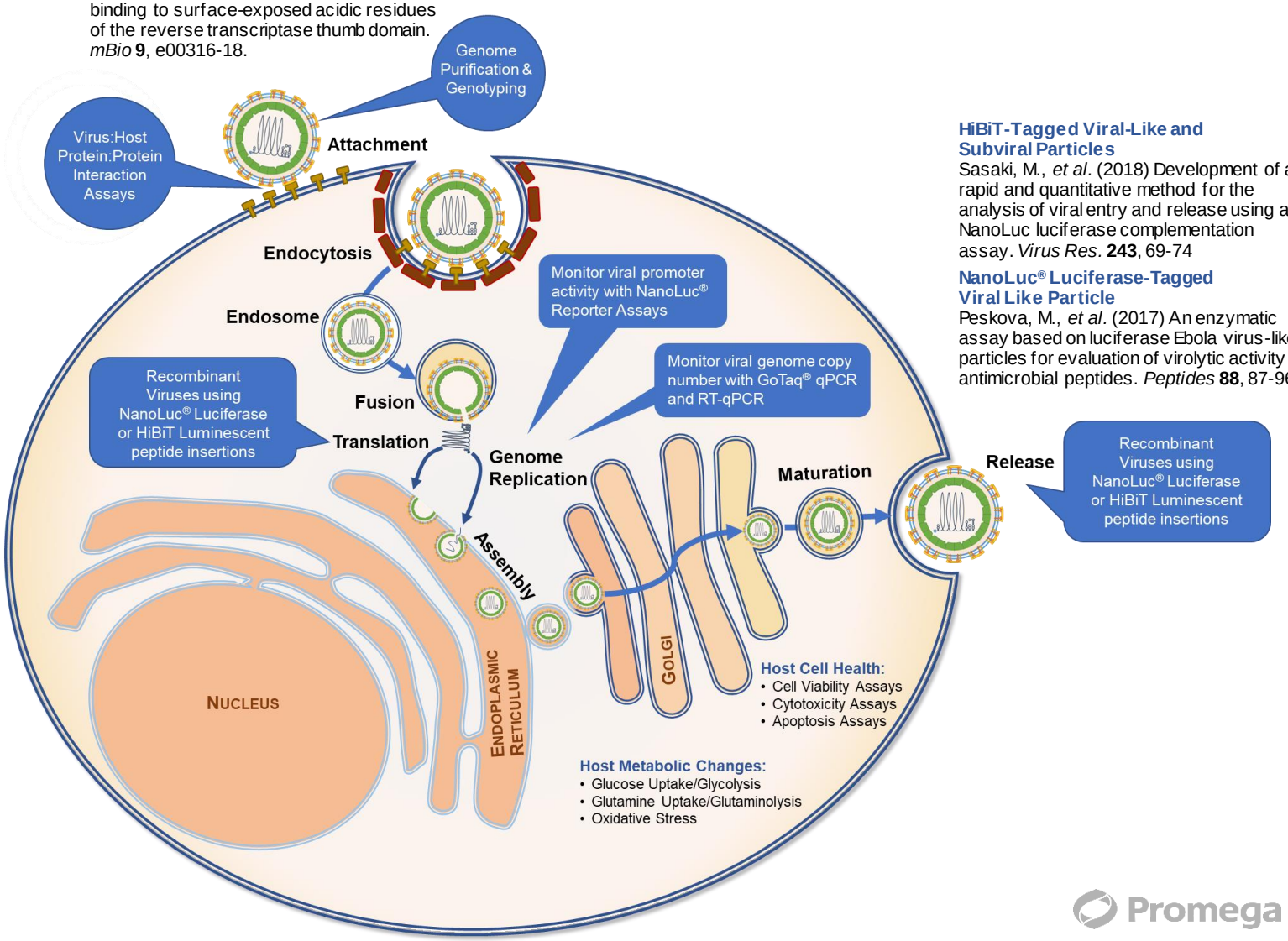
+ ssRNA virus (Hepatitis C, Dengue, Japanese Encephalitis, Bovine Viral Diarrhea Viruses)

Tamura, T., *et al*. (2018) Characterization of recombinant *Flaviviridae* viruses possessing a small reporter tag. *J. Virol*. **92**, e01582-17.

NanoBRET™ Technology for Protein:Protein Interactions (BRET)

Miyakawa, K., *et al*. (2017) The tumour suppressor APC promotes HIV-1 assembly via interaction with Gag precursor protein. *Nature Comm*. **8**, 14259.

Rawle, D.J., *et al*. (2018) HIV-1 uncoating and reverse transcription require eEF1A binding to surface-exposed acidic residues of the reverse transcriptase thumb domain. *mBio* **9**, e00316-18.

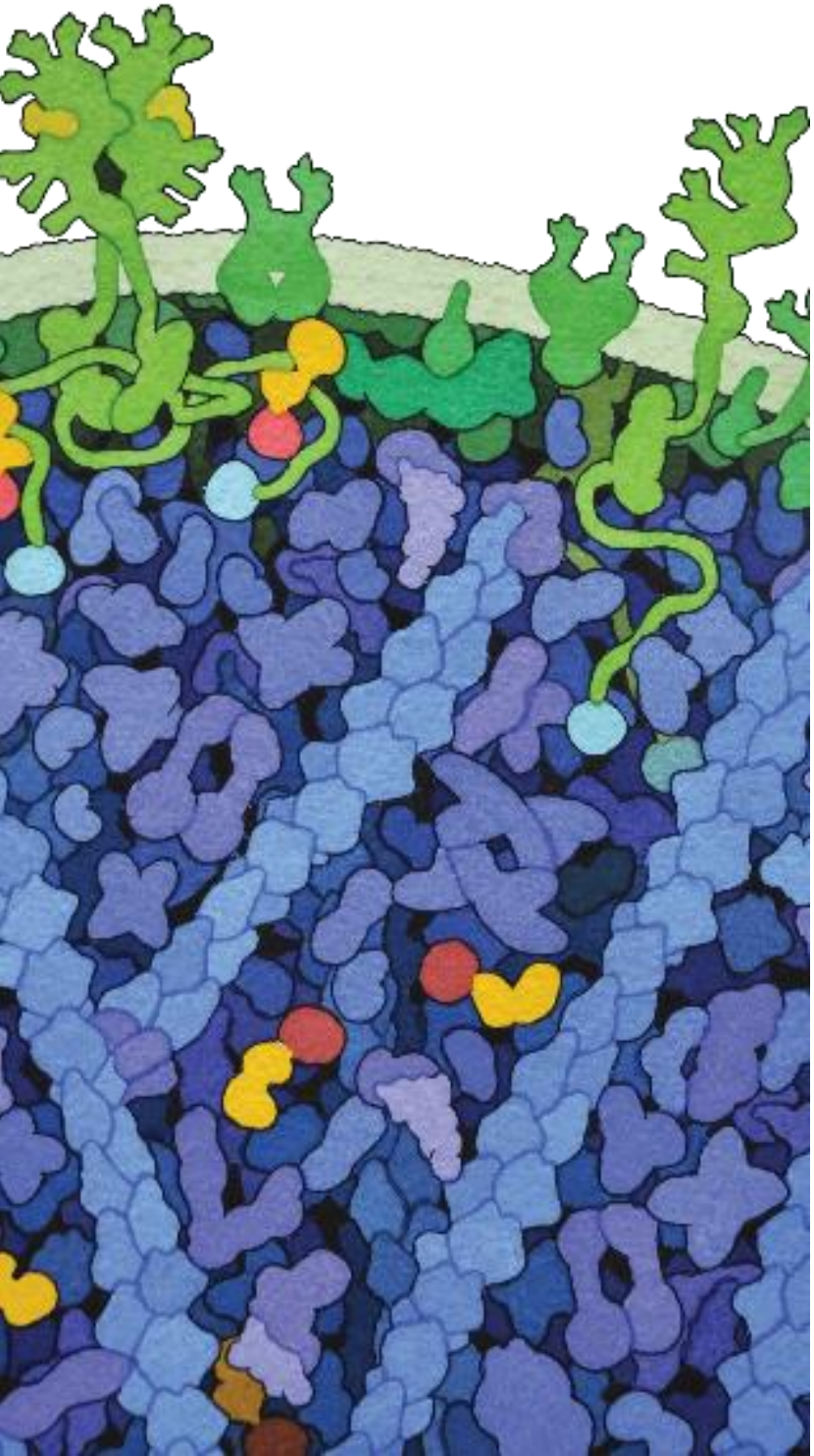


HiBiT-Tagged Viral-Like and Subviral Particles

Sasaki, M., *et al*. (2018) Development of a rapid and quantitative method for the analysis of viral entry and release using a NanoLuc luciferase complementation assay. *Virus Res*. **243**, 69-74

NanoLuc® Luciferase-Tagged Viral Like Particle

Peskova, M., *et al*. (2017) An enzymatic assay based on luciferase Ebola virus-like particles for evaluation of virolytic activity of antimicrobial peptides. *Peptides* **88**, 87-96.



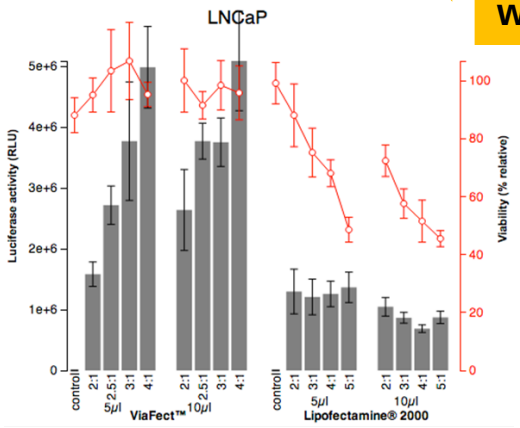
Transfection and Related Reagents

ViaFect™ Transfection Reagent

Efficiently Introduce DNA Into a Wide Variety of Cell Lines

Maintain cell health during transfection

Learn more & see data from 30+ cell lines at: **www.promega.com/viafect**

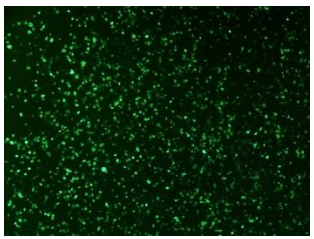


ViaFect™ Transfection Reagent allows high-efficiency transfection of a wide range of cell types without compromising cell viability and provides an easy-to-use protocol that gives superior performance with minimal optimization. Now you can design the right assay in the right cells to model the biology you are studying..

- Simple protocol with minimal optimization
- Use the best cell line for your research
- Keep your cells viable

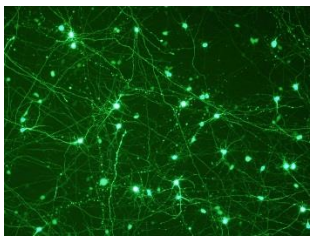
Product	Cat. #	Size
ViaFect™ Transfection Reagent	E4981	1ml
	E4982	5 x 1ml

Create assays in difficult cell lines including differentiated induced pluripotent stem cells.



iCell® Hepatocytes

iCell® Cardiomyocytes



iCell® DopaNeurons

Transfection and imaging of GFP transfected into differentiated IPS cells using ViaFect™ Reagent. Cellular Dynamics iCell® Hepatocytes were transfected with a 6:1 ratio of ViaFect™:DNA. iCell® Cardiomyocytes used a 2:1 ratio. Both cell lines were imaged 1 day post-transfection. iCell® DopaNeurons were transfected with a 4:1 ratio and imaged 3 days post-transfection. Data courtesy of Cellular Dynamics International.

Example Citations:

Lee, J.A., *et al.* (2018) Activation of the Nrf2 signaling pathway and neuroprotection of nigral dopaminergic neurons by a novel synthetic compound KMS99220. *Neurochem. Intl.* **112**, 96-107.

Park, S.-W., *et al.* (2018) The expression of the embryonic gene Cripto-1 is regulated by OCT4 in human embryonal carcinoma NCCIT cells. *FEBS Lett.* **592**, 24-35.

Robaszkiewicz, A., *et al.* (2018) PARP1 facilitates EP300 recruitment to the promoters of the subset of RBL2-dependent genes. *Biochem. Biophys. Acta* **1861**, 41-53.

Ayroldi, E., *et al.* (2018) Long glucocorticoid-induced leucine zipper regulates human thyroid cancer cell proliferation. *Cell Death Dis.* **9**, 305.

Ji, H., *et al.* (2018) Zinc-finger nucleases induced by HIV-1 Tat excise HIV-1 from the host genome in infected and latently infect cells. *Mol. Ther. Nucl. Acids* **12**, 67-74..

Zaremba-Czogalla, M., *et al.* (2018) A novel regulatory function of CDKN1A/p21 in TNFα-induced matrix metalloproteinase 9-dependent migration and invasion of triple-negative breast cancer cells. *Cell. Signal*. **47**, 27-36..

Liu, Z., *et al.* (2018) Transcription of blunt snout bream (*Megalobrama amblycephala*) *HIF3α* and its localization in the nucleus under both normoxic and hypoxic conditions. *Biochem. Biophys. Res. Comm.* **500**, 443-9.

Tsuchiya, M., *et al.* (2018) Cell surface flip-flop of phosphatidylserine is critical for PIEZO1-mediated myotube formation. *Nat. Comm.* **9**, 2049.

Xu, F., *et al.* (2018) Type III interferon-induced CBFβ inhibits HBV replication by hijacking HBx. *Cell. Mol. Immunol.* DOI 10.1038/s41423-018-0006-2.

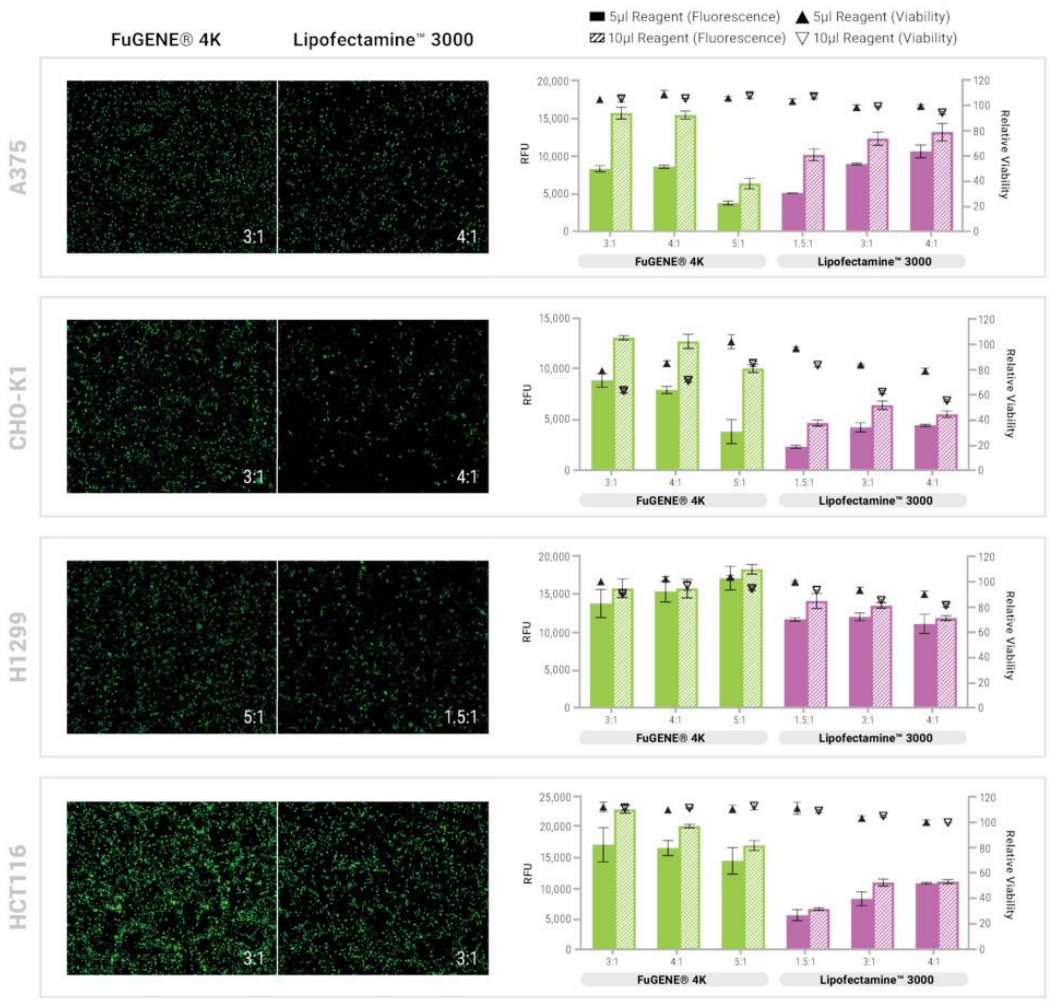
Promega

FuGENE® 4K Transfection Reagent

Best-in-Class DNA Transfection Reagent for Routine and Challenging Cell Lines

2022 New Product

Unbeatable Transfection Efficiency with Minimal Effect on Cell Health



FuGENE® 4K is a 100% synthetic, multicomponent transfection reagent designed for the delivery of DNA into challenging and routine mammalian cell lines. Improving on previous generations of FuGENE® technology, FuGENE® 4K efficiently delivers DNA into a wide range of cell lines while providing the gentle, low-toxicity transfection you've come to expect from FuGENE® reagents..

Product	Cat. #	Size
FuGENE® 4K Transfection Reagent	E5911	1ml
	E5912	5 x 1ml

Example Citations:

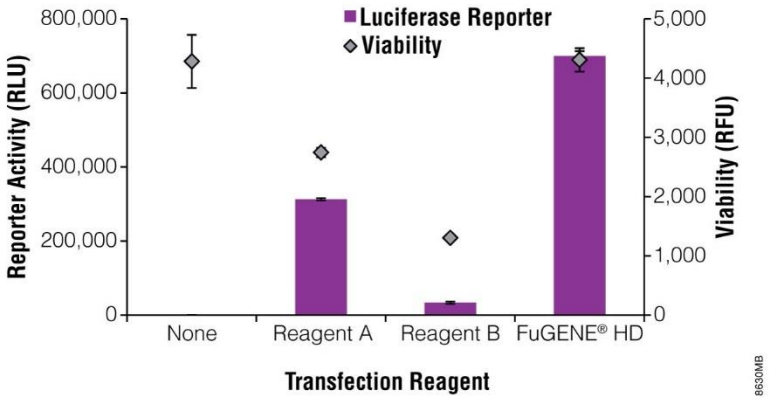
Schwalm, M.P., et al (2023) Tracking the PROTAC degradation pathway in living cells highlights the importance of ternary complex measurement for PROTAC optimization. Preprint. *doi: https://doi.org/10.1101/2023.01.11.523589*

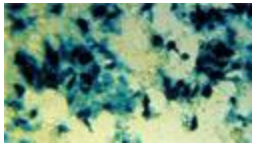
FuGENE® 4K reagent delivers improved DNA transfection performance while minimizing impact on cell health. A375, CHO-K1, H1299 and HCT116 cell lines were transfected with a GFP expression construct using FuGENE® 4K or Lipofectamine™ 3000 (ThermoFisher Scientific) with varying reagent:DNA ratios. After 48 hours, cells in a clear-bottom plate were measured for total GFP fluorescence. Cell viability was measured on a duplicate plate using the CellTiter-Glo® Luminescent Cell Viability Assay.

FuGENE® HD Transfection Reagent

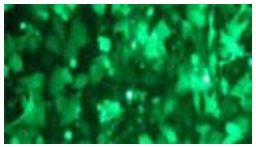
High Efficiency, Low Toxicity Transfection for Challenging Applications

High efficiency and low toxicity

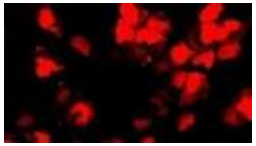




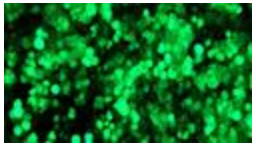
Transfection of stem cells
FuGENE® HD Transfection Reagent was used to transfect mouse embryonic stem cells cultured on an extracellular matrix.



Use with cancer models
FuGENE® HD Transfection Reagent lets you work in your cancer model. The online protocol database includes over 25 cancer models.



Cellular imaging
See more of your cells! High expression and low toxicity enhance your imaging experiments with the FuGENE® HD reagent.



Protein production
Get more from your cells! No animal components and compatible with defined media.

FuGENE® HD Transfection Reagent is a trusted delivery system for NanoLuc®, NanoBRET™, NanoBiT™, pGL4, HaloTag®, and GloSensor™ DNA constructs into a variety of cell lines. Transfection can be performed in 100% serum to mimic in vivo conditions. There is no need to remove serum or culture medium, and there is no washing or changing of medium after adding the reagent/DNA complex.

Product	Cat. #	Size
FuGENE® HD Transfection Reagent	E2311	1ml
	E2312	5 x 1ml

FuGENE® 6 Transfection Reagent

High Efficiency, Low Toxicity Transfection Reagent for Common Cell Types

FuGENE® 6 Transfection Reagent is a nonliposomal formulation designed to transfect plasmid DNA into a wide variety of cell lines with high efficiency and low toxicity. The protocol does not require removal of serum or culture medium and does not require washing or changing of medium after introducing the reagent/DNA complex.

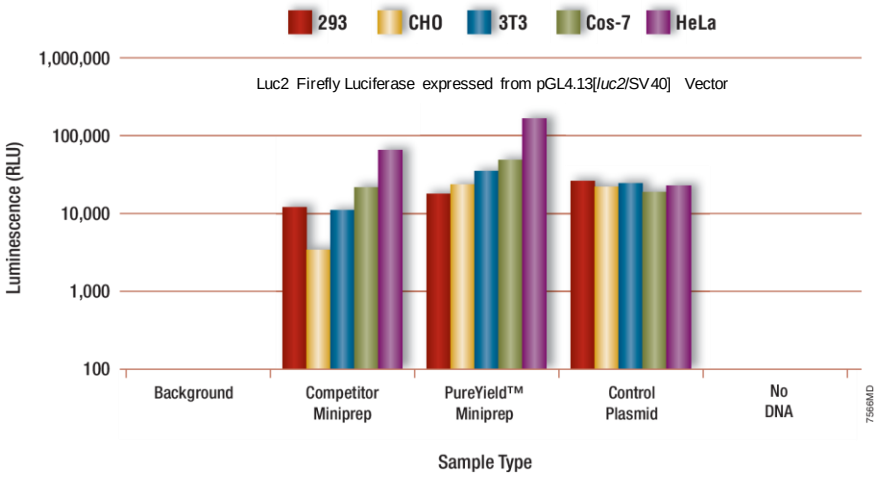
- **More Biologically Relevant**: Very low toxicity; less impact on biology.
- **Simple Protocol**: No culture changes; less variability; compatible with serum.
- **Effective in Many Cell Types**: Used in thousands of publications.
- **Ideal for Use with Luciferase Assays**: More expression; sensitive results.

Product	Cat. #	Size
FuGENE® 6 Transfection Reagent	E2691	1ml
	E2692	5 x 1ml
	E2693	0.5ml

PureYield™ Plasmid Purification Systems

Manual, transfection-grade plasmid at the mini, midi and maxi scale

High-Quality Plasmid for Transfecting Common Cell Lines



Eluator™ Vacuum Elution Device and Vac-Man® Manifold are reusable and speed PureYield™ Midipreps and Maxipreps purifications.

Miniprep System: Up to 15µg of Transfection-Ready Plasmid from 3ml Cultures

- Rapid, 10-minute protocol
- No tedious, gravity-drip columns or post-elution alcohol precipitation

Product	Cat. #	Size
PureYield™ Plasmid Miniprep System	A1223	100 preps
	A1222	250 preps

Midiprep System: Up to 200µg of Transfection-Ready Plasmid DNA from 50ml Cultures

- 30-minute, vacuum-based protocol
- No tedious, gravity-drip columns or post-elution alcohol precipitation

Product	Cat. #	Size
PureYield™ Plasmid Midiprep System	A2492	25 preps
	A2495	100 preps
	A2496	300 preps

Midiprep System: Up to 1mg of Transfection-Ready Plasmid from 250ml Cultures

- One-hour, vacuum-based protocol
- No tedious, gravity-drip columns or post-elution alcohol precipitation

Product	Cat. #	Size
PureYield™ Plasmid Maxiprep System	A2392	25 preps
	A2393	100 preps

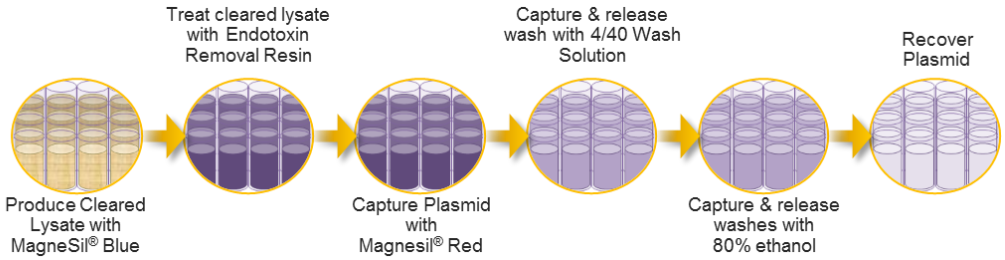
Accessory Items

Product	Cat. #	Size
Vac-Man® Laboratory Vacuum Manifold	A7231	Each
Eluator™ Vacuum Elution Device	A1071	4 each

Wizard MagneSil Tfx™ System
Transfection-Quality Plasmid DNA in 96-Well Format

Turn your liquid handler into a transfection-grade plasmid purifier.

Paramagnetic Particle-based Chemistries



Provides a simple and reliable method for rapid isolation of transfection-quality plasmid DNA using silica-coated paramagnetic particles in a 96-well, high-throughput format without centrifugation or vacuum manifolds. .

- **Automation Friendly**: Methods available for all liquid handlers including particle movers Learn more at: www.promega.com/automethods.
- **Rapid Results**: Process a full plate in ~1 hour (96-well head) to ~1½ hours (8-channel).

Product	Cat. #	Size
Wizard MagneSil Tfx™ System	A2380	4 x 96 preps
Custom Wizard MagneSil Tfx™ System quantities and volumes available. Please contact COD@promega.com for more information.		

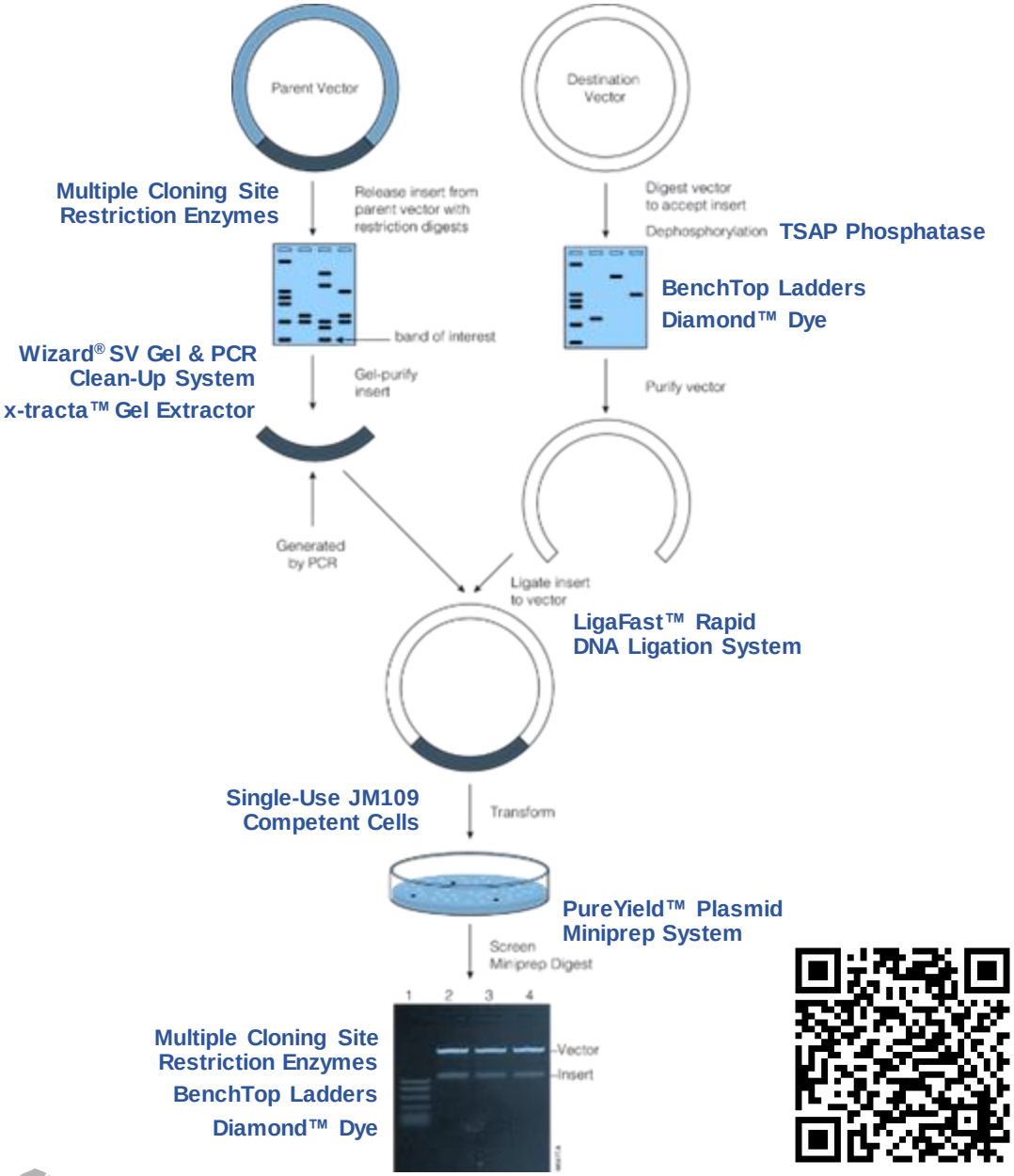
You Are Not Alone!

Promega's Field Support Scientists (FSS) in combination with our internal engineering and applications support teams work seamlessly to help develop a custom, optimized high throughput solution either on your liquid handler or recommend an automation solution to meet your specific needs.
Contact FSS@promega.com

Promega

Standard MCS Subcloning Tools

Quality molecular biology tools to aid standard protein reporter constructs



https://bit.ly/2FBWMiG

Product	Cat. #	Size
TSAP Thermosensitive Alkaline Phosphatase	M9910	100u
LigaFast™ Rapid DNA Ligation System	M1821	30 rxns
Wizard® SV Gel and PCR Clean-Up System	A9281	50 preps
x-tracta™ Gel Extractor	A2121	25/pack
PureYield™ Plasmid Miniprep System	A1223	100 preps
Single-Use JM109 Competent Cells, >10^8cfu/µ	L2005	20 x 50µl
BenchTop 1kb DNA Ladder	G7541	100 lanes
BenchTop 100bp DNA Ladder	G8291	50 lanes
Diamond™ Nucleic Acid Dye	H1181	500µl
Protein Reporter Multiple Cloning Site Restriction Enzymes*		
Apa I (10u/µl)	R6361	5,000u
Bam HI (10u/µl)	R6021	2,500u
Bgl II (10u/µl)	R6081	500u
Eco RI (12u/µl)	R6011	5,000u
Eco RV (10u/µl)	R6351	2,000u
Hind III (10u/µl)	R6041	5,000u
Nhe I (10u/µl)	R6501	250u
Not I (10u/µl)	R6431	200u
Pvu I (2-10u/µl)	R6321	100u
Sac I (10u/µl)	R6061	1,000u
Sac II (10u/µl)	R6221	500u
Sgf I (8-12u/µl)	R7103	250u
Xba I (10u/µl)	R6181	2,000u
Xho I (10u/µl)	R6161	3,000u

* Available from Promega. Vectors may have more unique restriction sites available.

Flexi® Vector Subcloning Tools

Quality molecular biology tools to create Flexi® Vector Protein Reporter Constructs

Flexi® Vector Basics

Clone once, transfer directionally to many vectors for many applications

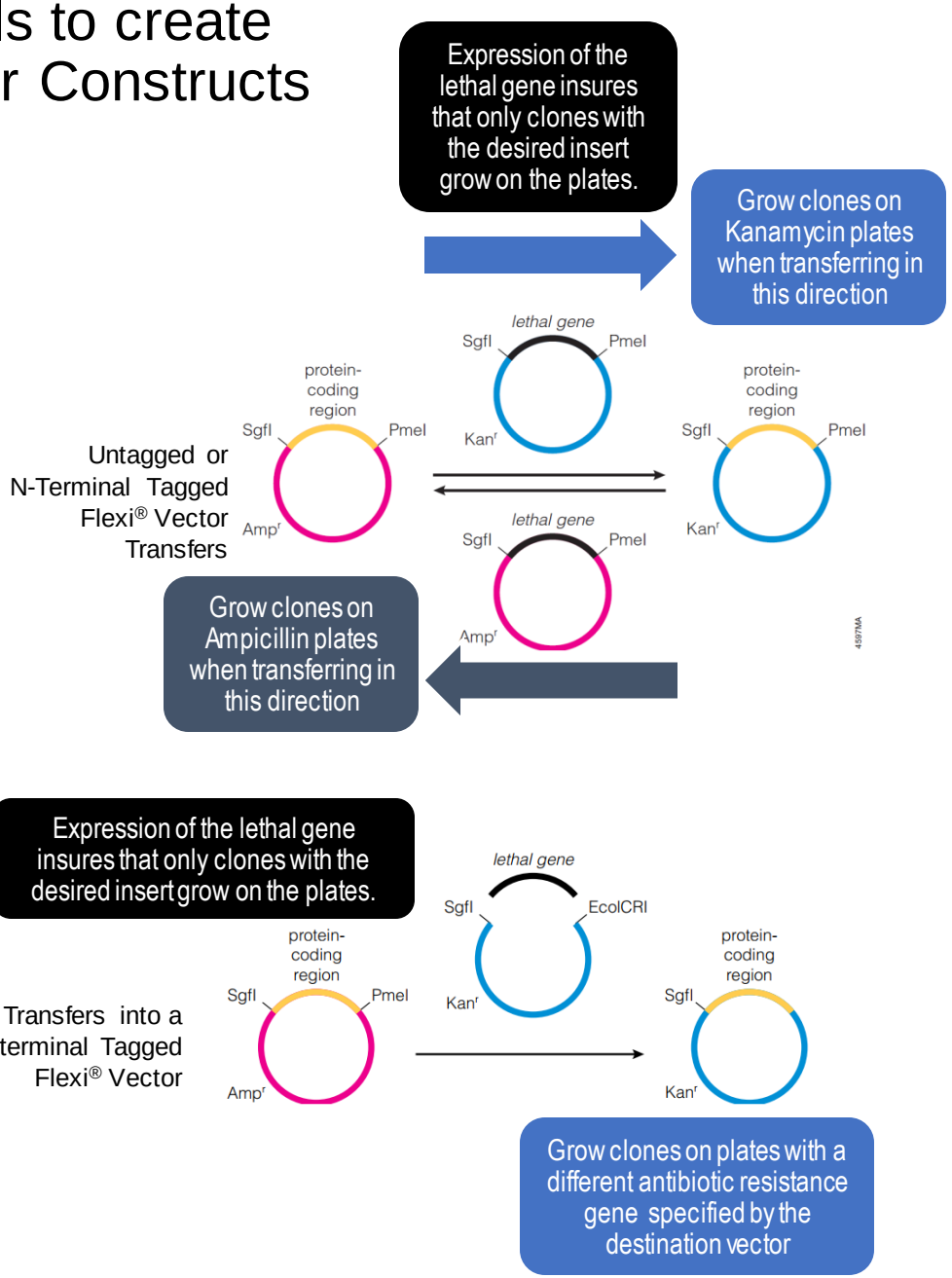
Uses rare-cutting restriction enzymes, changes in antibiotic resistance plus a lethal gene to reduce cloning background

Versatility: You can choose between a variety of initial applications (e.g., bacterial protein, mammalian, or cell-free protein expression) and then transfer to others as required.

Time Savings: Efficient transfer allows for direct use of recombinant clones, minimizing time wasted screening background colonies.

Enhanced Productivity: Adaptable to high-throughput formats for large screening projects.

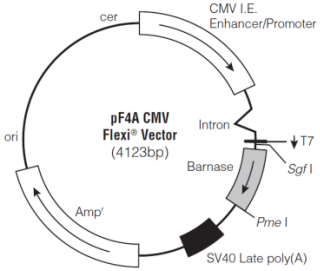
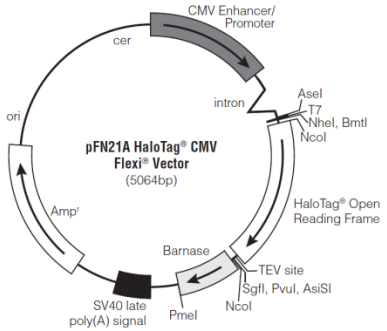
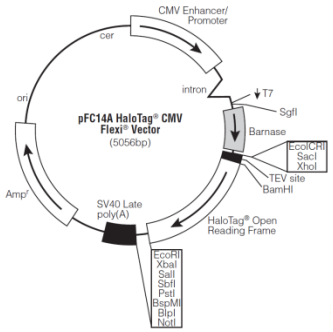
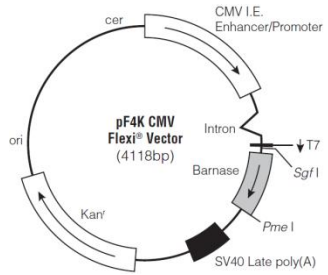
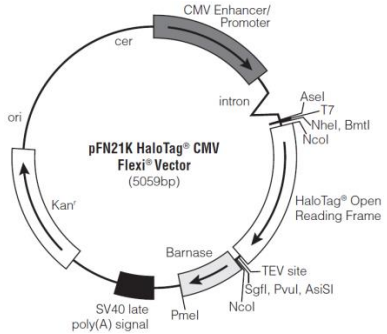
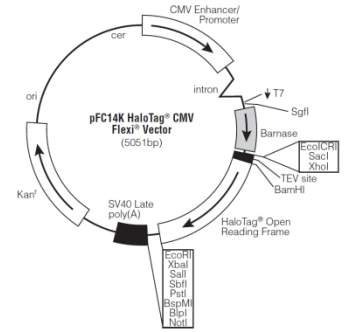
Easy Access: No licensing fees or complicated transfer restrictions.



Promega

Flexi® Vector Basics

Deciphering the plasmid name

	Untagged Vector	Tag at N-terminus Vector	Tag at C-terminus Vector
Ampicillin Resistant			
Kanamycin Resistant			

pF4K

- indicates a Flexi® Vector
- indicates antibiotic resistance

pFN21K

- indicates a N-terminal fusion tag

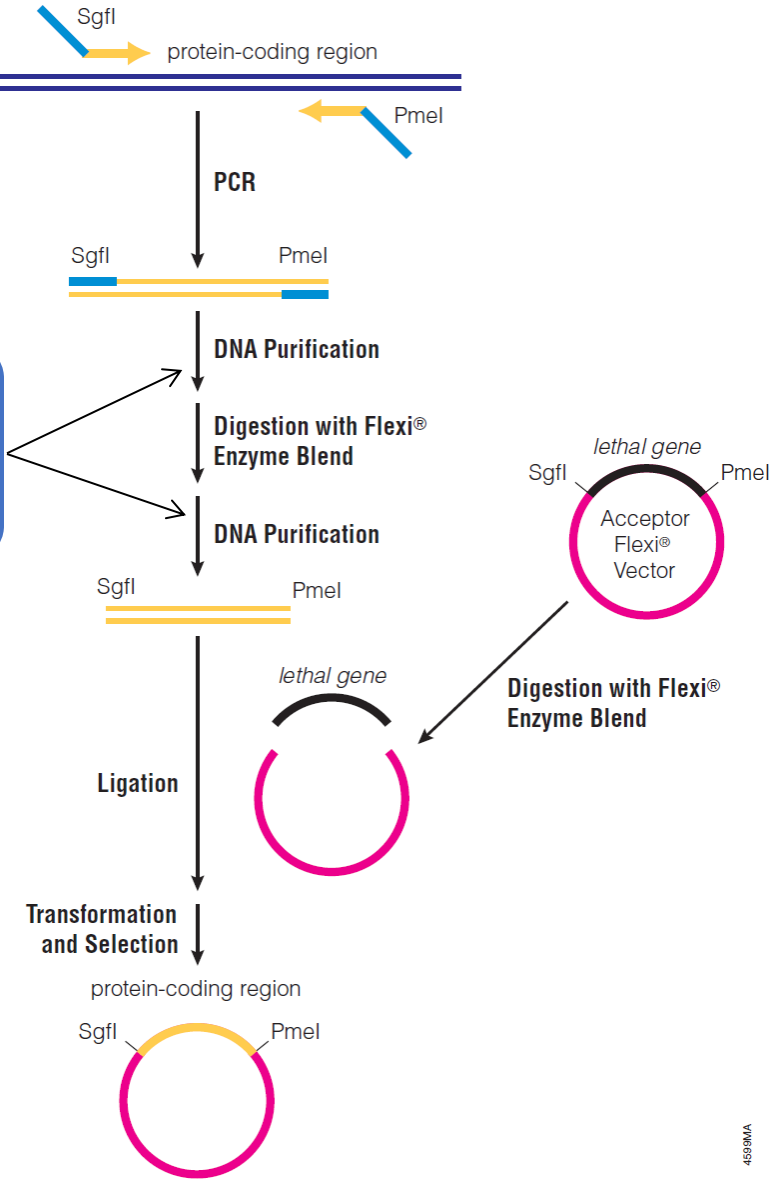
pFC14K

- indicates a C-terminal fusion tag

Flexi® Vector Selector available through on-line catalog

Getting a Sequence into a Flexi® Vector

Flexi® System, Entry/Transfer



Wizard® SV Gel & PCR Clean-Up System included for entry reactions

Design primers for the Flexi Cloning System using online Promega tool or contact Technical Services for assistance

The Flexi® Vector System is a directional cloning method for protein-coding sequences. It is based on two rare-cutting restriction enzymes, SgfI and PmeI, and provides a rapid, efficient and high-fidelity way to transfer protein-coding regions without the need to resequence. Once an ORF is cloned, conveniently transfer the inserts into a variety of vectors designed for multiple applications. No licensing fees or complicated transfer restrictions needed.

All Flexi® Vectors carry the lethal barnase gene, which is replaced by the DNA fragment of interest and acts as a positive selection for the successful ligation of the insert.

Unlike site-specific recombination vector systems, the Flexi® Vector Systems do not require appending multiple amino acids to the amino or carboxy termini of the protein of interest. In addition, the systems do not require an archival entry vector, and most applications allow direct entry into the vector suited to the experimental design.

C-terminal Flexi® Vectors allow expression of C-terminal-tagged proteins. While these vectors can act as acceptors of protein-coding regions flanked by SgfI and PmeI, they lack a PmeI site and contain a different blunt-ended site, EcoICRI. This joined sequence cannot be removed from the C-terminal Flexi® Vectors and transferred to other Flexi® Vectors.

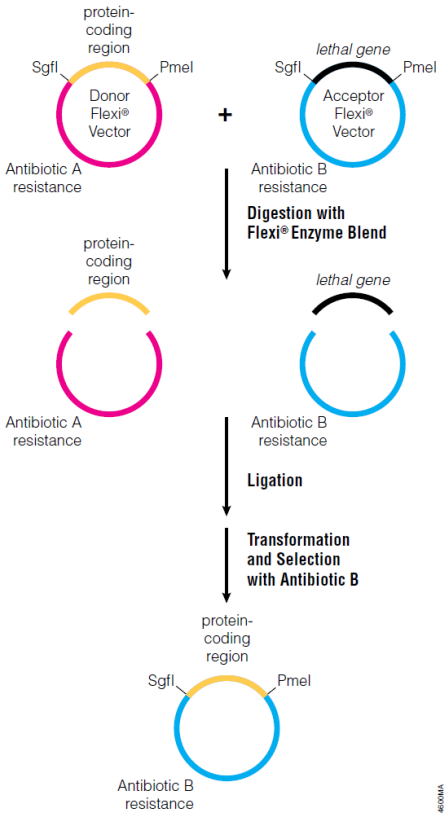
Product	Cat. #	Size
Flexi® System, Entry/Transfer Flexi® Enzyme Blend (SgfI/PmeI) and buffer T4 DNA Ligase and Flexi® Ligase Buffer Wizard® SV Gel and PCR Clean-Up System Nuclease-free Water	C8640	5 entry 20 transfer
10X Flexi® Enzyme Blend	R1851	25µl
	R1852	100µl
Carboxy Flexi® Enzyme Blend (Sgf I & EcoICR I)	R1901	50µl

Transferring Inserts between Flexi® Vectors
Two Flexi® Transfer Systems

Transfer from Flexi® Vector to Flexi® Vector

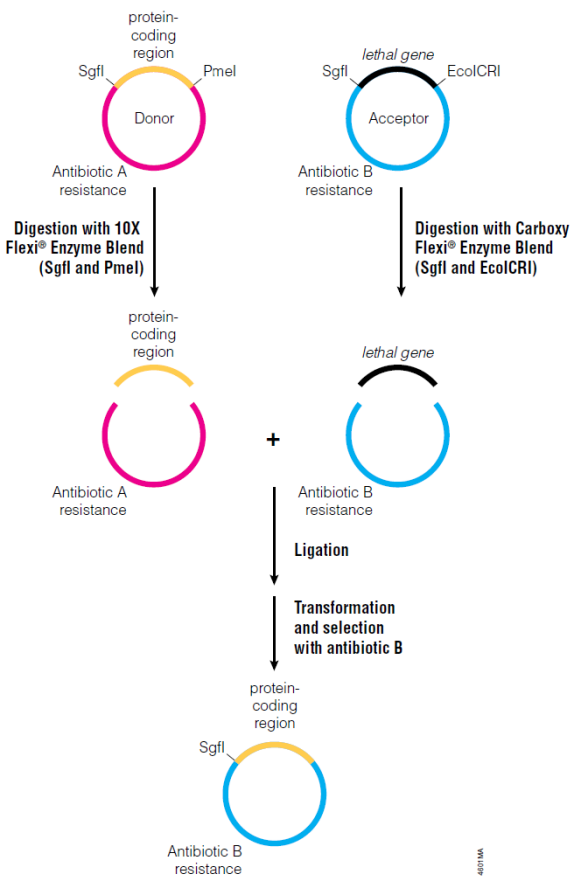
Bi-Directional

N-Tagged ⇄ N-Tagged
N-Tagged ⇄ Untagged
Untagged ⇄ Untagged



Uni-Directional

N-Tagged → C-Tagged
Untagged → C-Tagged



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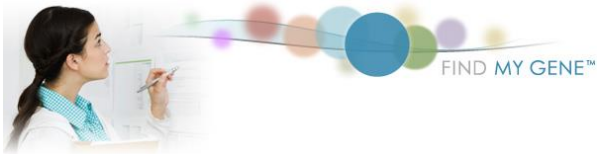
Product	Cat. #	Size
Flexi® System, Transfer Flexi® Enzyme Blend (SgfI & PmeI) and Flexi® Digest Buffer T4 DNA Ligase and Flexi® Ligase Buffer Nuclease-free Water	C8820	100 Transfers
Carboxy Flexi® System, Transfer Carboxy Flexi® Enzyme Blend (SgfI & EcoICRI) Flexi® Enzyme Blend (SgfI & PmeI) Flexi® Digest Buffer T4 DNA Ligase and Flexi® Ligase Buffer Nuclease-free Water	C9320	50 Transfers
10X Flexi® Enzyme Blend	R1851	25µl
	R1852	100µl
Carboxy Flexi® Enzyme Blend (SgfI & EcoICRI)	R1901	50µl

Validated Flexi® Vector ORF Clones to Speed Your Research

N-terminal Tagged HaloTag® and Untagged Human clones

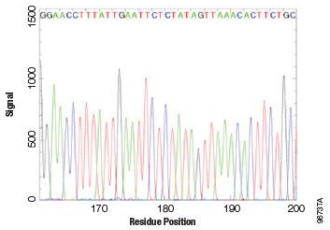
Features	HaloTag® ORF Collection (HaloTag® @ N-Terminus)	Native ORF Collection (Untagged)
Size of Collection	>8,500	>6300
Validations:		
Sequence Confirmed (100%)	✓	✓
Insert Retrievable (SgfI & PmeI) (99.8% of clones)	✓	✓
Expression Confirmed (99.8% of clones)	✓	
Localization Confirmed (81.4% of clones)	✓	
Quantity Delivered	100ng	100ng
Order to Delivery Time	3-4 weeks	3-4 weeks



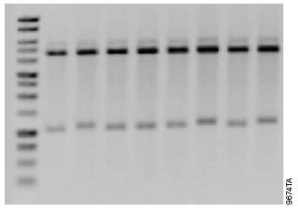
Find Your Gene and Ordering Information at:
www.promega.com/FindMyGene
or use the promega.com search engine

Need assistance?
Contact Technical Services by phone, e-mail or live chat:
www.promega.com/support

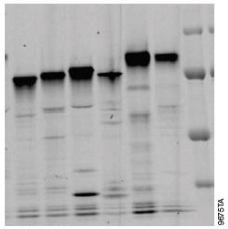
Sequence validated
5' and 3' ends sequenced



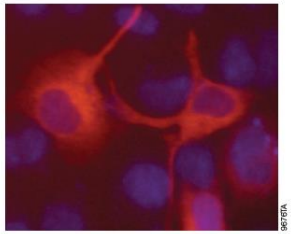
Insert validated
Confirmed size of clone insert



Expression validated
Clones expressed in HEK293 cells



Cell Imaging validated
Confirmed protein localization with HaloTag® TMR ligand



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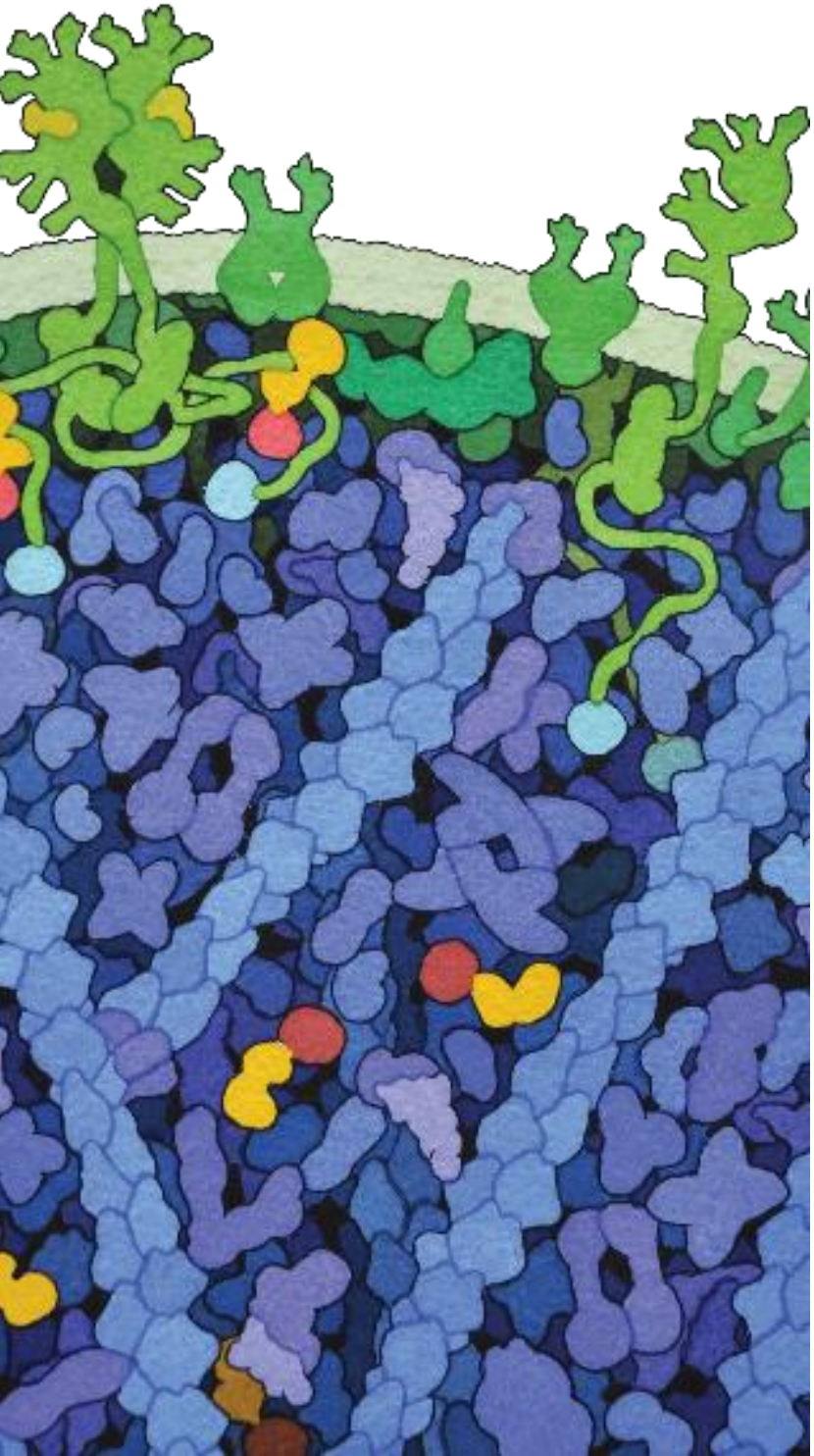


Detection Instruments

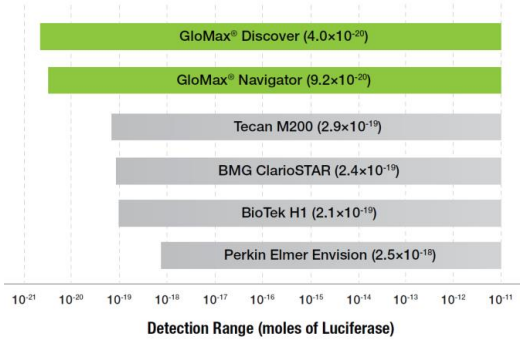
Learn more about the GloMax® Family of Microplate Readers

www.promega.com/glomax

GloMax® Detection Systems
The Readers Designed for Your Assays

Easy-to-Use, Luminescence Plate Reader

The **GloMax® Navigator** is an easy-to-use microplate luminometer integrated with Promega chemistries for superior luminescence assay performance. The GloMax® Navigator comes ready to use—simply unpack it, plug it in and begin your experiments. You can also interpret results using integrated data analysis software.



Multimode Plate Reader with Maximum Flexibility

The **GloMax® Explorer** is a high-performance multimode detection instrument that allows you to get up and running quickly, generating the data you need from your experiments. Simply unpack it, plug it in, and begin your experiments. You can also interpret your results using integrated data analysis software. Developed with Promega reagents to provide a simple means of detecting advanced chemistries, the GloMax® Explorer measures luminescence, fluorescence intensity and visible absorbance. The GloMax® Explorer can be used as a standalone instrument or integrated into your high-throughput automated workflow.

Easy-to-Use Microplate Reader with Advanced Detection Capabilities

GloMax® Discover is a ready-to-use multimode plate reader developed with Promega reagent chemistries to provide a simple means of detecting luminescence, fluorescence and absorbance. This advanced plate reader provides flexible use of filters and includes **high-performance luminescence, fluorescence, UV-Visible absorbance, BRET and FRET, two-color filtered luminescence and kinetic measurement capabilities**. GloMax® Discover can be used as a standalone plate reading instrument or integrated into high-throughput automated workflows. Results are easy to interpret using integrated data analysis software.

GloMax® Instruments are Designed for Sensitivity

Learn more about the GloMax® Family of Microplate Readers

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GloMax® Detection Systems
The Readers Designed for Your Assays

Instrument	Luminescence	Fluorescence	Vis Absorbance	UV Absorbance	BRET/FRET
GloMax® Discover Model GM3000	✔	✔	✔	✔	✔
GloMax® Explorer Model GM3500	✔	✔	✔		
GloMax® Explorer Model GM3510	✔	✔			
GloMax® Navigator Model GM2000	✔				

Will read all Promega Bioluminescent Assays

Will read all Promega Fluorescent Assays

Will read all Promega Colorimetric Assays

230nm, 260nm, 280nm & 320nm

Optimized for NanoBRET™ Assays

96-well Dual-Injector options for each instrument

Cat. # GM3030 shown for GloMax® Discover or Explorer Models and can be added after purchase. Navigator must be ordered with or without dual-injectors

Ask for a GloMax® Demo in your Lab!

PC Tablet interface on all plate readers

GloMax® Navigator is a workhorse 96-well luminometer

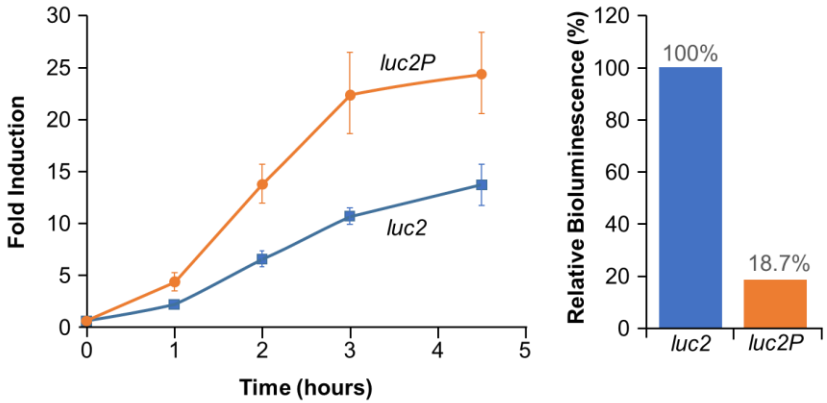
GloMax® Discover & Explorer read 6- through 384-well plates

GloMax® Discover & Explorer are Automation Ready

Luminometer Choice Does Make a Difference

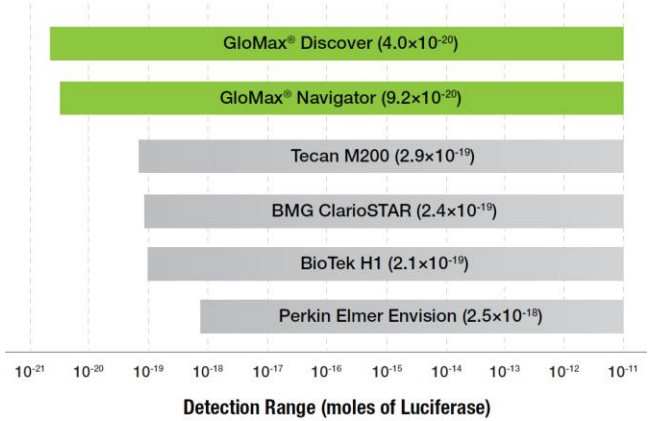
Balancing brightness with responsiveness

Some Assays Use Specific Luciferase Genes to Maximize Responsiveness, Not Brightness

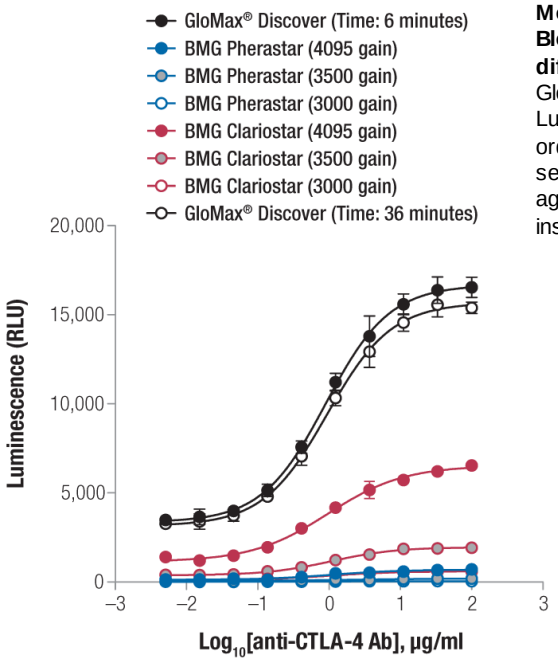


The *luc2P* gene shows greater fold induction but produces about 20% of the light output of the *luc2* gene. Luciferase genes were inserted behind a cAMP response element/minimal promoter construct and treated with isoproterenol.

GloMax® Instruments are Designed for Sensitivity

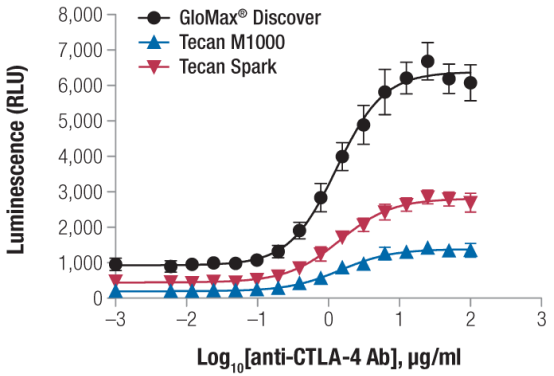


Your Instrument Sensitivity Can Determine Success or Failure of an Assay



Monitoring the signal of the CTLA-4 Blockade Bioassay (Cat. # JA3001) on different instruments. Results were read on GloMax® Discover 6 minutes after Bio-Glo™ Luciferase Reagent addition then read in order on the instruments listed with the gain settings indicated. Finally, the plate was read again on the same GloMax® Discover instrument 30 minutes later.

Instrument	EC_{50}
GloMax® Discover (6 minutes)	0.8996
BMG Pherastar (4095 gain)	1.122
BMG Pherastar (3500 gain)	2.085
BMG Pherastar (3000 gain)	0.7653
BMG Clariostar (4095 gain)	0.9672
BMG Clariostar (3500 gain)	1.079
BMG Clariostar (3000 gain)	1.083
GloMax® Discover (36 minutes)	0.9402



Instrument	EC_{50}
GloMax® Discover	1.3
Tecan M1000	1.471
Tecan Spark	1.442

14526MA

Additional Resources

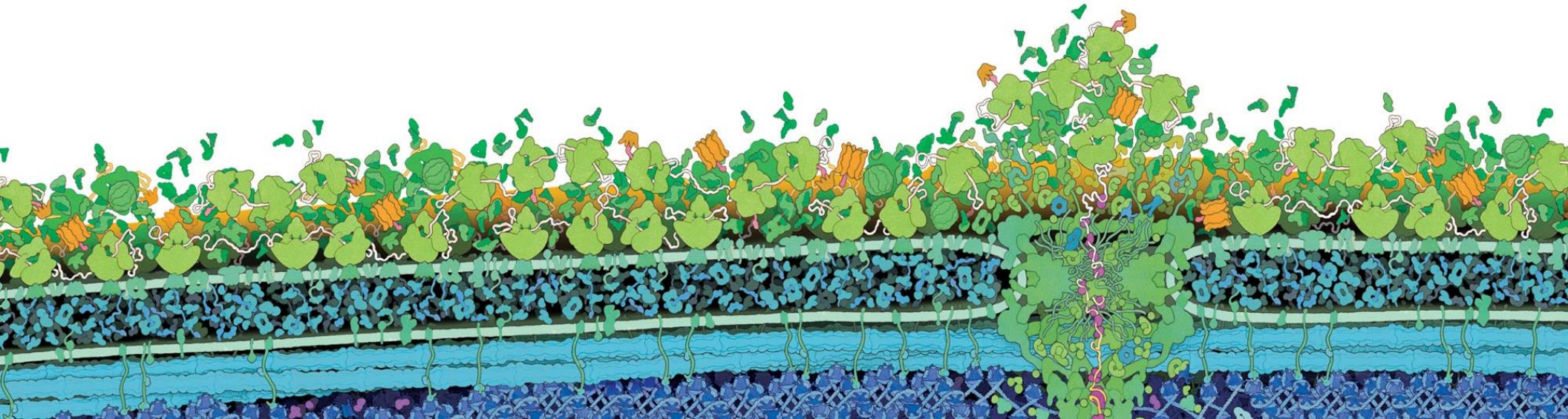
Recent Webinar Recordings

Custom & Bulk Ordering

Tailored R&D Solutions

Integration Support

Questions Answered



Webinar Recordings…

Bioluminescence: Past, Present, Future

https://tinyurl.com/3da8r76v

Thomas Machleidt, PhD
Research Director
Promega Corporation

Originally Broadcast:
September 13, 2021

Ask the Experts: Practical Guidance for Designing Successful Targeted Protein Degradation Assays

https://tinyurl.com/yjbr2zza

Kristin Riching, PhD
Sr Research Scientist
Promega Corporation

Originally Broadcast:
September 23, 2020

Applications of CRISPR Knock-in Tagging for Studying Endogenous Protein Dynamics

https://tinyurl.com/yck3wksn

Chris Eggers, PhD
Sr Research Scientist
Promega Corporation

Originally Broadcast:
September 14, 2021

Understanding Kinase Target Engagement in Live Cells

https://tinyurl.com/468ce79j

Matt Robers, MS
Sr Research Scientist
Promega Corporation

Originally Broadcast:
September 15, 2021

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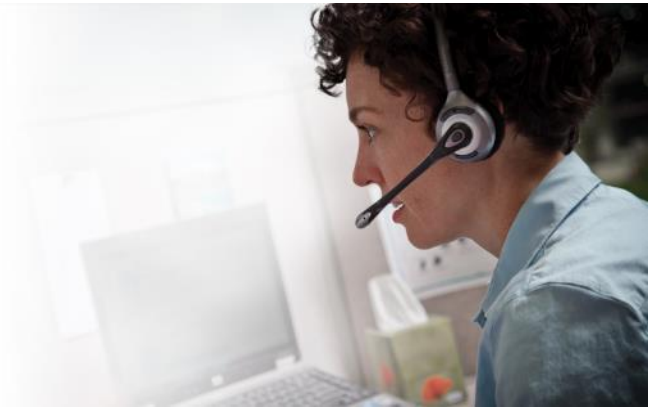

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