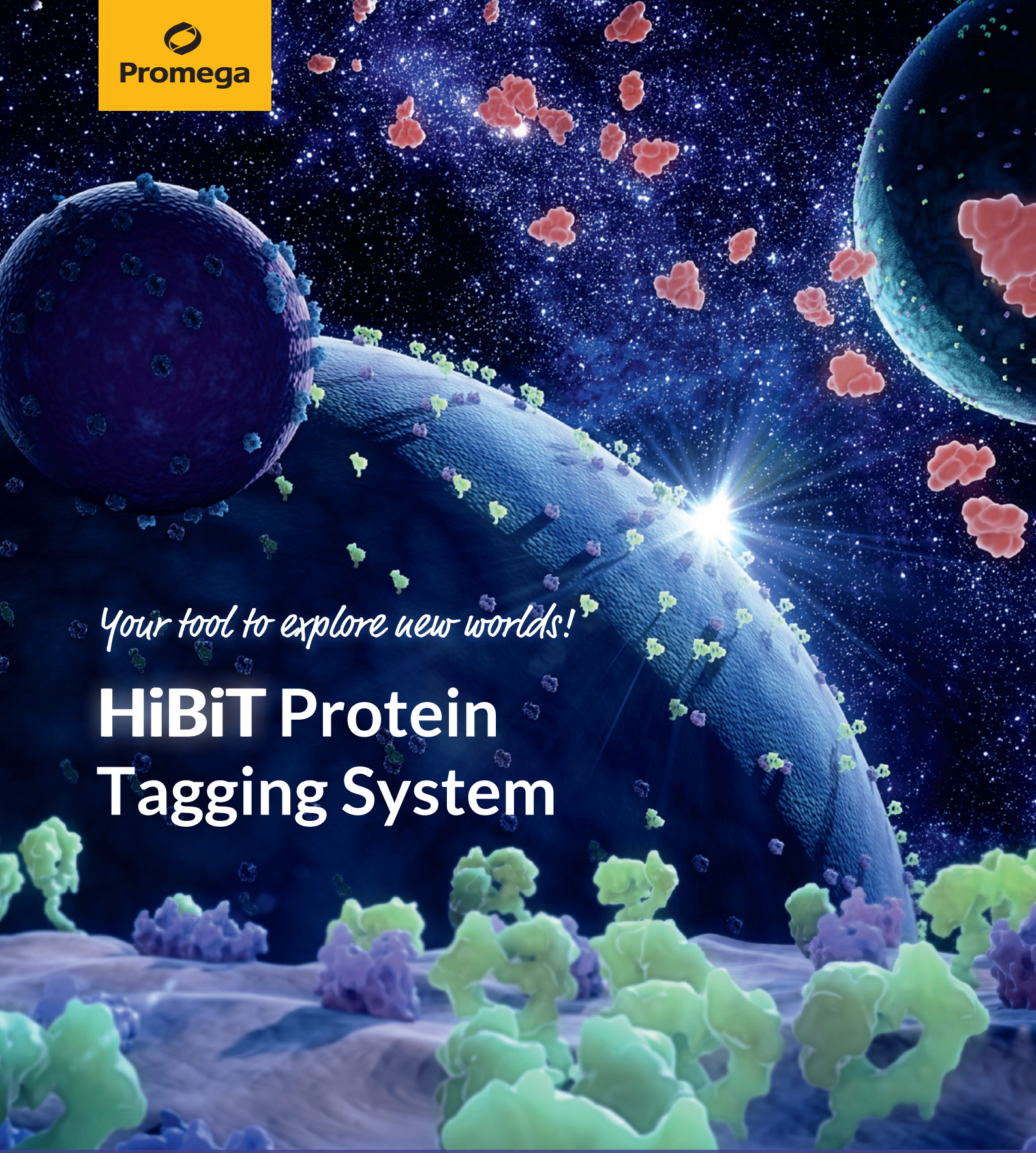
Promega

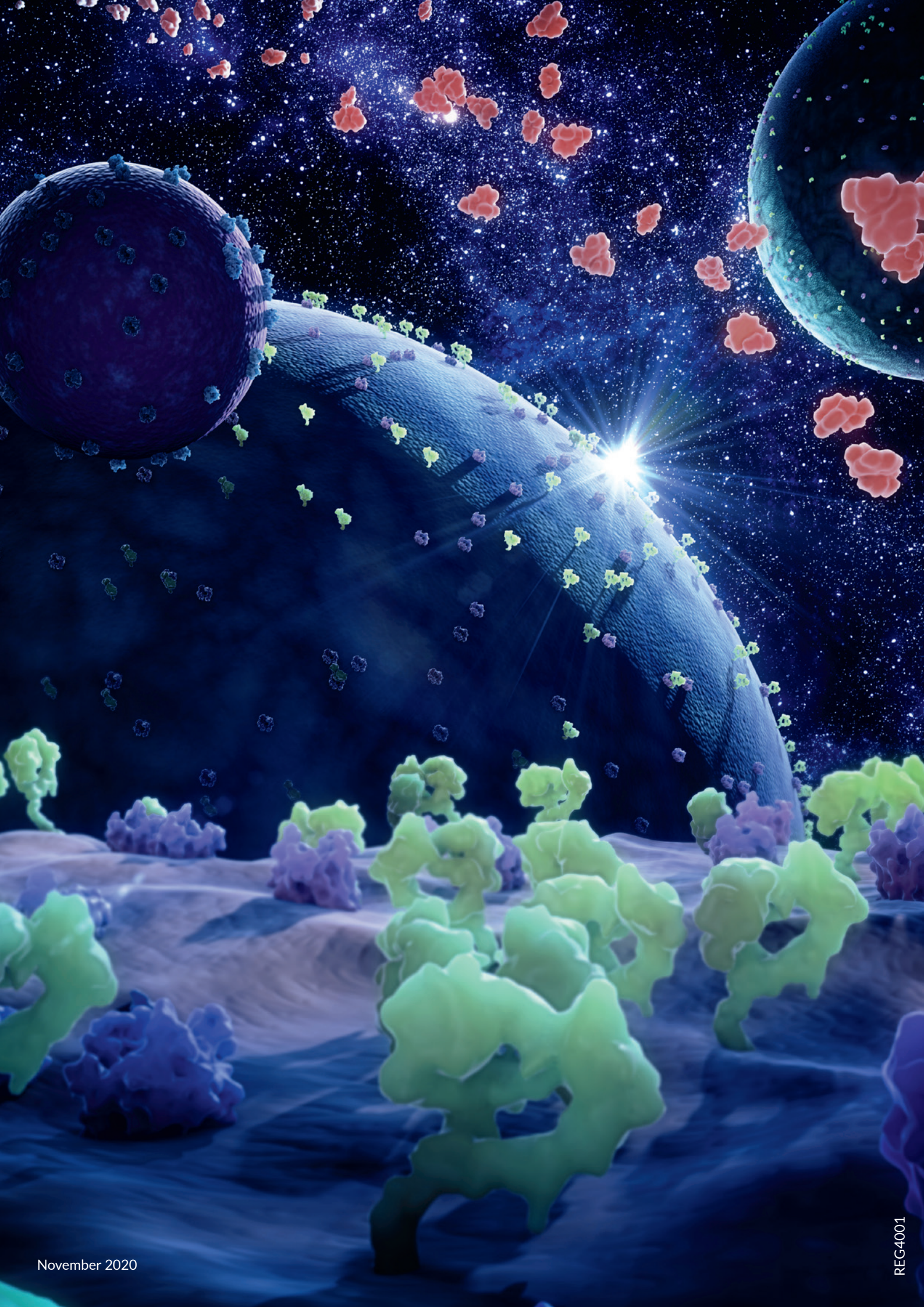


Your tool to explore new worlds!

HiBiT Protein Tagging System

Advanced Protein Quantification

Expression Analysis | Protein Stability | Protein Degradation |
Autophagy | Membrane Receptor Trafficking |
Viral & Bacterial Infection | Target Cell Killing



November 2020

REG4001

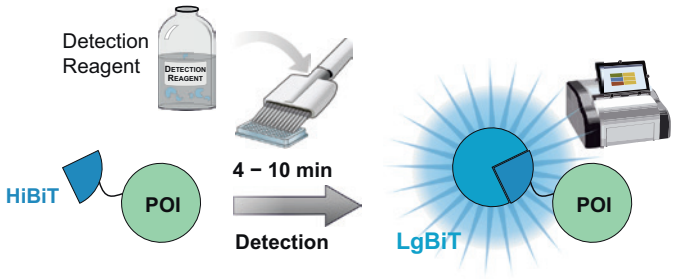
HiBiT Protein Tagging System

brings the power of bioluminescence to protein analysis

The *HiBiT Protein Tagging System* facilitates the development of simple, highly sensitive protein assays to measure changes in protein abundance or localization. HiBiT simplifies protein tagging in live cells, providing a streamlined, antibody-free detection protocol that requires only a luminometer for signal quantification. With the sensitivity to measure endogenous expression levels and the convenience of a single reagent-addition step, HiBiT technology opens up a universe of possibilities for researchers studying protein biology.

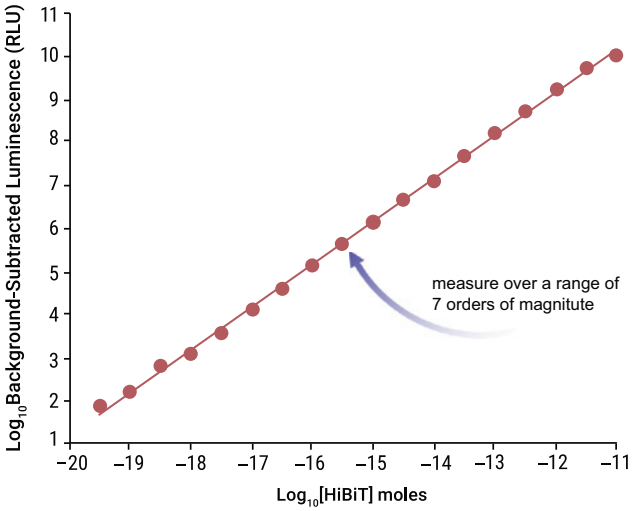
Principle

HiBiT (High BiT) is an 11 amino acid peptide tag that can be attached to any protein-of-interest (POI) and detected in 10 minutes or less using simple bioluminescent reagents. The detection reagent contains an inactive luciferase subunit, LgBiT (Large BiT), which rapidly binds to HiBiT to produce a highly active luciferase enzyme.



Sensitive Protein Quantification For Endogenous Biology

The broad linear dynamic range accurately quantifies HiBiT-tagged proteins regardless of their expression level. With a detection limit of less than 10^{-19} moles, the *HiBiT Detection Systems* support quantification of even low abundance proteins at endogenous levels of expression.



Key Publications

Schwinn, M.K. *et al.* (2020) A simple and scalable strategy for analysis of endogenous protein dynamics. *PSci Rep.* **10**(1):8953

Boursier, M.E. *et al.* (2020) The luminescent HiBiT peptide enables selective quantitation of G protein-coupled ligand engagement and internalization in living cells. *J. Biol. Chem.* **295**(15):5124–5135

Riching, K.M. *et al.* (2018) Quantitative Live-Cell Kinetic Degradation and Mechanistic Profiling of PROTAC Mode of Action. *ACS Chem Biol.* **13**(9):2758–2770

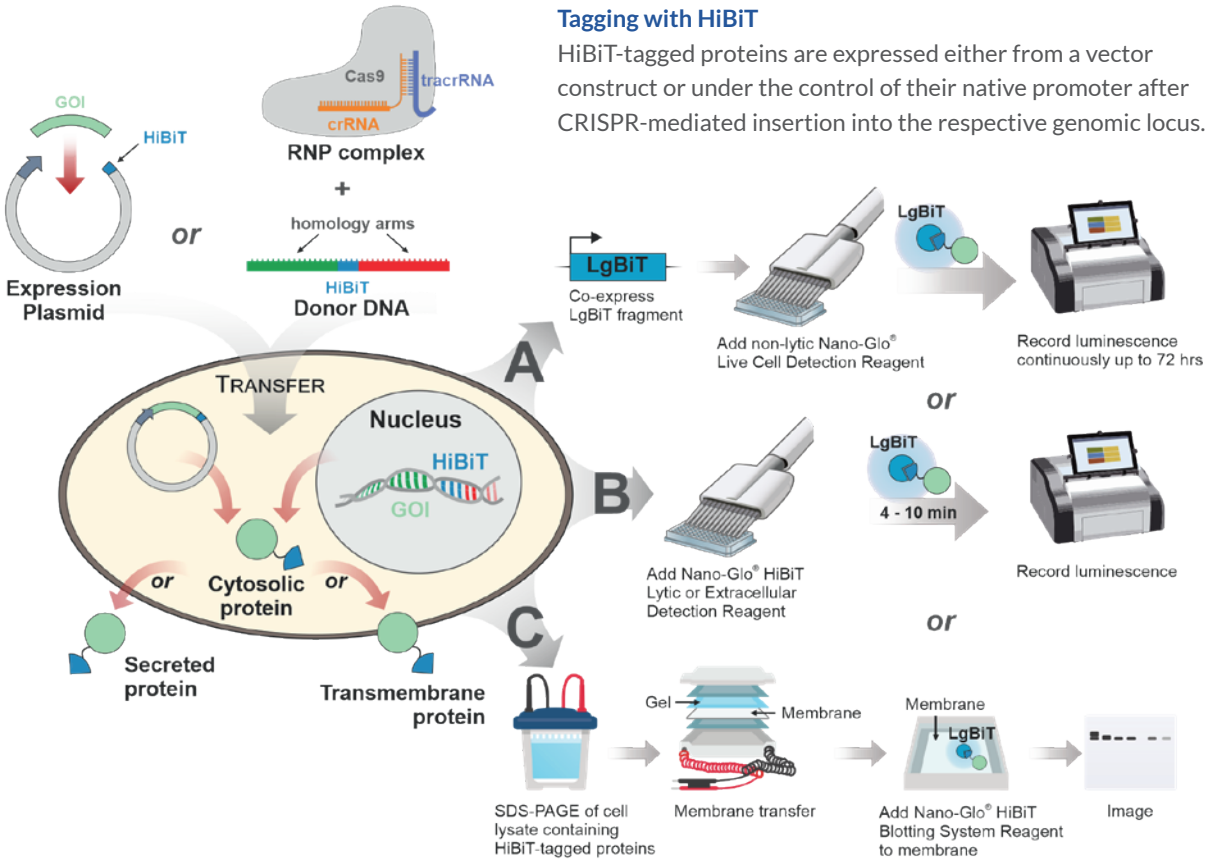
Sasaki, M. *et al.* (2018) Development of a rapid and quantitative method for the analysis of viral entry and release using a NanoLuc luciferase complementation assay. *Virus Res.* **243**:69–79

Schwinn, M.K. *et al.* (2018): CRISPR-Mediated Tagging of Endogenous Proteins with a Luminescent Peptide. *ACS Chem Biol.* **13**(2):467–474

Oh-Hashi, K. *et al.* (2017) Application of a novel HiBiT peptide tag for monitoring ATF4 protein expression in Neuro2a cells. *Biochem Biophys Rep.* **12**:40–45

Rouault, A. A. J. *et al.* (2017) Regions of MRAP2 required for the inhibition of orexin and prokineticin receptor signaling. *Biochim Biophys Acta.* **1864**:2322–2329

HiBiT Tagging & Quantification Workflow



Tagging with HiBiT

HiBiT-tagged proteins are expressed either from a vector construct or under the control of their native promoter after CRISPR-mediated insertion into the respective genomic locus.

Quantification of HiBiT-tagged proteins: The amount of HiBiT-tagged proteins can be easily quantified using "add-and-read" assays without the need of antibodies. **(A)** Transient or stable co-expression of the LgBiT subunit in combination with one of the non-lytic Nano-Glo® substrates enables real-time kinetic studies for up to 72 hrs. **(B)** The lytic detection reagent quantifies the total amount of HiBiT-tagged proteins, whereas the extracellular detection reagent detects cell-surface or secreted proteins. **(C)** By use of the HiBiT blotting reagent the size of HiBiT-tagged proteins is rapidly determined on a conventional western blot membrane.

A perfect match – HiBiT & CRISPR/Cas9

Either *do-it-yourself* with a cloning-free protocol for rapid success or choose from our growing collection of assay-ready CRISPR-edited cell lines for maximum convenience!

~ 10 days

1. Design and order CRISPR components ← *start here with DIY protocol*
2. Assemble RNP complex and donor DNA and deliver into cells
3. Verify genomic edit with HiBiT detection reagents

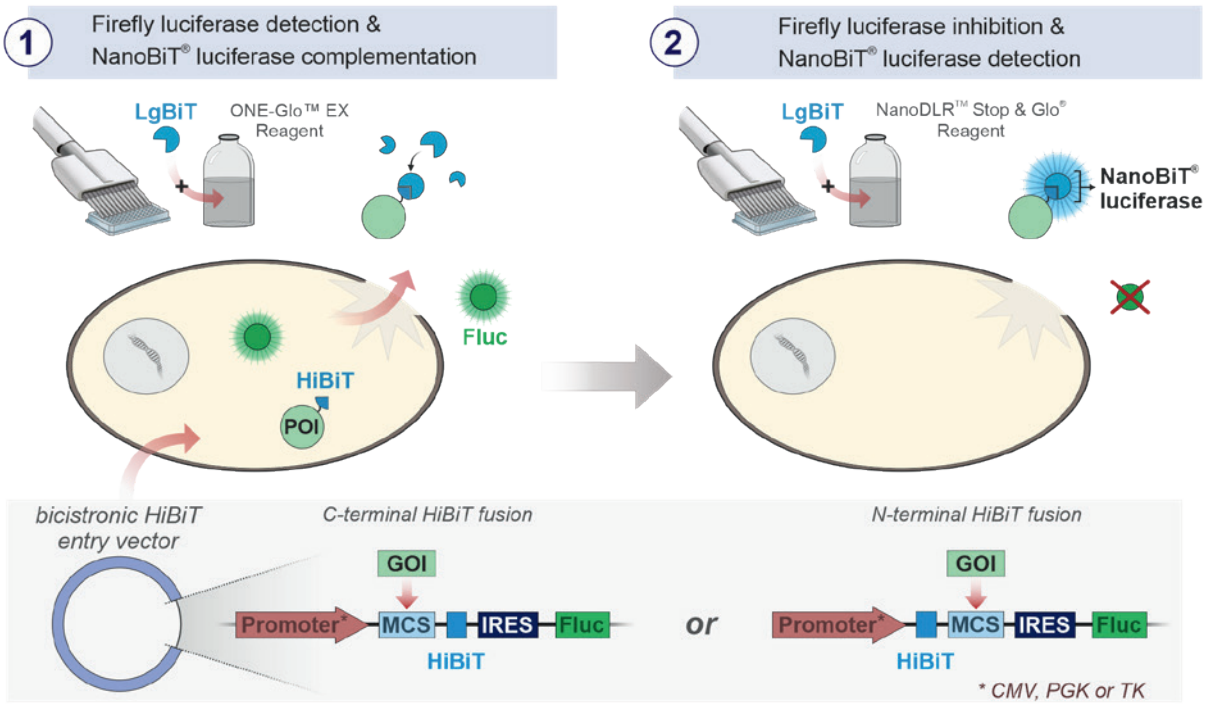
Experiment Perform experiment directly with edited cell pool or proceed with clone isolation ← *start here with ready-to-use edited cells*

Learn more about your options: **www.promega.com/hibit-crispr-knock-in**

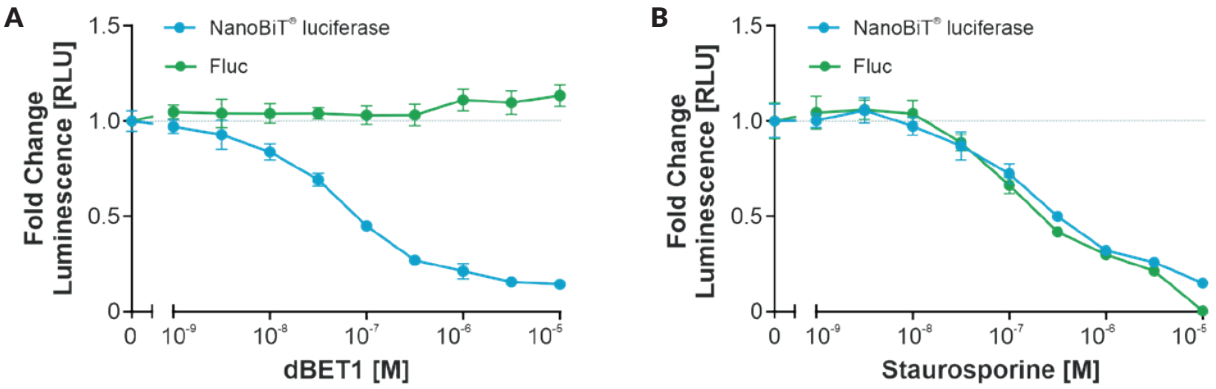
Control for Specificity by Normalization to Control Reporter

HiBiT NanoDLR™

The *Nano-Glo® HiBiT Dual-Luciferase® Reporter System* enables multiplexed measurement of HiBiT-tagged proteins and firefly luciferase (Fluc) in the same well. This can improve data quality by minimizing or eliminating experimental variability from such factors as transfection efficiency, cell number, cell viability, temperature, or measurement time. When measuring regulated degradation of a HiBiT-tagged protein of interest (POI), the assay can help distinguish specific effects from global changes in protein expression levels.



The coding sequence of the POI (GOI) is cloned into a multiple cloning site (MCS) with either an N- or C-terminal HiBiT fusion. Following the stop codon for the HiBiT fusion protein is an IRES sequence that enables constitutive co-expression of non-fused Fluc from the same mRNA. In the first step **(1)**, Fluc is quantified after addition of *ONE-Glo™ EX Reagent* supplemented with LgBiT Protein. This lyses cells, provides the Fluc substrate, and converts the HiBiT tag into the NanoBiT® luciferase. In the second step **(2)**, *NanoDLR™ Stop & Glo® Reagent* is added to the sample, quenching the Fluc signal and providing the NanoBiT® substrate.

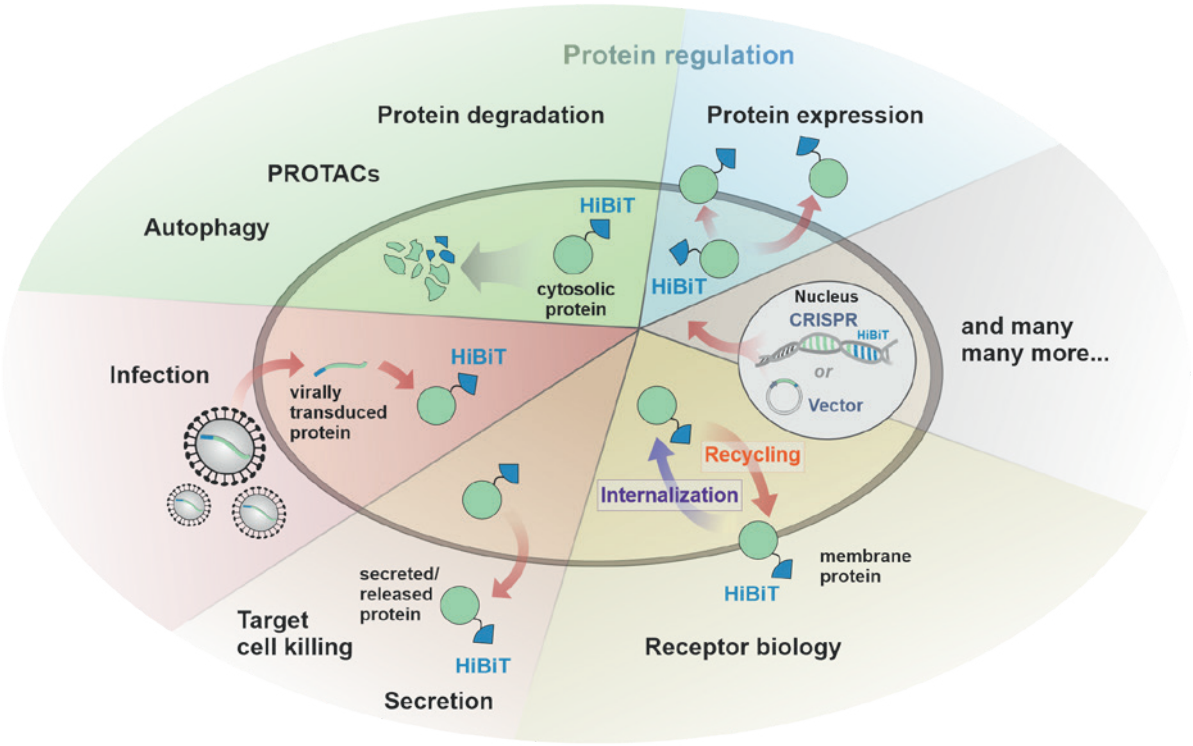


HEK293 cells were transiently transfected with a TK-driven bicistronic expression construct for HiBiT-BRD4 and Fluc. **(A)** Treatment of cells with dBET1, a PROTAC compound targeting BRD4 leads to specific degradation of HiBiT-BRD4 but not the Fluc control. **(B)** Treatment with the toxic compound staurosporine, on the other hand, shows a non-specific decrease in the signals from both proteins.

Applications of the HiBiT Protein Tagging System

The *HiBiT Protein Tagging System* opens up a universe of possibilities for researchers in the world of functional gene and protein analysis. It is a straightforward tool for building new assays to interrogate cellular protein biology with a bioluminescent protein tag. Optimized protocols and assay reagents enable you to easily establish cell-based assays for your individual protein of interest.

Measure regulated protein expression, protein stability, targeted protein degradation, receptor internalization, target cell killing...



Features & Benefits

Small Tag Size (11 aa, 1.3 kDa)

- Reduces potential impact on fusion partner function
- Greatly facilitates CRISPR/Cas9 workflow for genomic knock-ins

Simple & Fast Detection Protocol

- Homogenous 1-step assay ("add-and-read")
- No antibodies and no washing steps required
- Suitable for High-Throughput Screening Assays

Sensitive & Quantitative

- Sub-attomole sensitivity enables measurement of proteins at endogenous levels
- Large linear detection range (> 7 logs)

Facilitates Endogenous Protein Assays with CRISPR/Cas9

- Study proteins under endogenous regulation
- No cloning required
- Optimized protocol available

Multiple Assay Formats

- Lytic endpoint format
- Live-cell format (intra- and extracellular) with an option for extended time course analysis

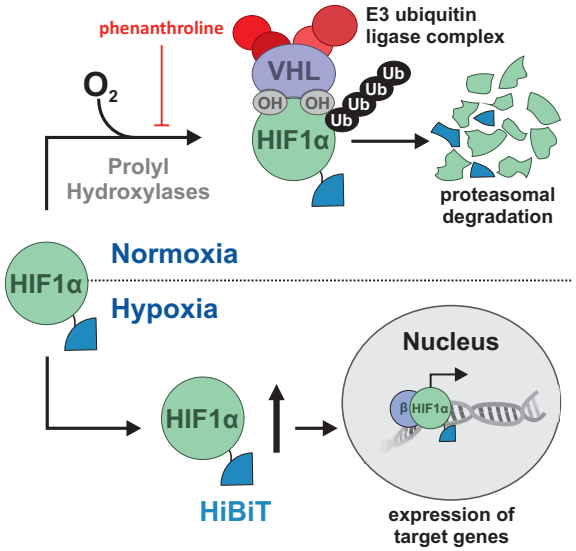
Endogeneous expression for highest response

Monitor Post-Translational Regulation of Proteins in Cells

The HiBiT Tagging Technology can be easily applied to monitor post-translational regulatory processes such as ubiquitin-mediated degradation as shown below for the transcription factor HiF1α. For this experiment the *Nano-Glo® HiBiT Lytic Detection System* was used to measure regulated changes in protein abundance.

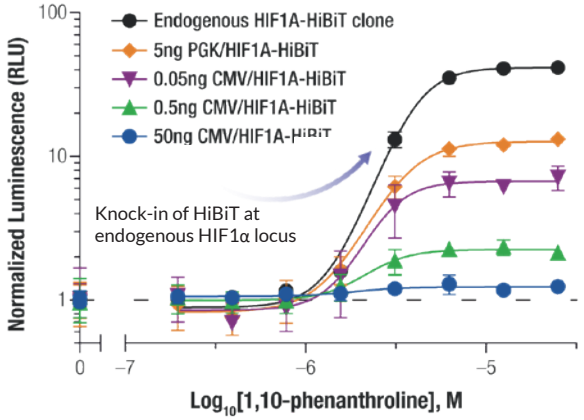
Stabilization of transcription factor HIF1α

The level of the constitutively expressed transcription factor HIF1α is controlled by the presence of molecular oxygen (O_2). Under normoxic conditions HIF1α becomes prolyl hydroxylated in an oxygen-dependent reaction by prolyl hydroxylases. This modification leads to the recruitment of the von-Hippel-Lindau (VHL) tumor suppressor and other components of the E3 ubiquitin ligase complex that marks HIF1α for proteasomal degradation by poly-ubiquitination. During hypoxia or upon chemical inhibition of prolyl hydroxylases with phenanthroline, HIF1α accumulates, dimerizes with the ß-subunit and drives the expression of HIF1α-responsive genes. By tagging HIF1α with HiBiT, this post-translational regulatory process can be easily measured.

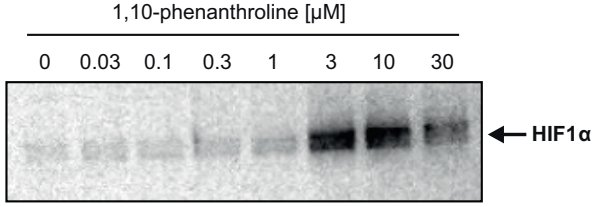


Quantification of hypoxia-induced stabilization of HIF1α at various expression levels

HIF1α-HiBiT was expressed in HeLa cells by either transient transfection of varying amounts of CMV or PGK-promoter-driven expression constructs or by CRISPR-mediated insertion of the HiBiT tag at the endogenous locus. Upon addition of the hypoxia mimetic 1,10-phenanthroline, HIF1α-HiBiT protein expression levels were quantified using the *Nano-Glo® HiBiT Lytic Detection System*. Maximal fold-response was detected for HIF1α-HiBiT expressed under endogenous regulatory conditions. Endogenous expression not only reduces artifacts related to overexpression but also maintains the proper stoichiometry with endogenous binding partners.



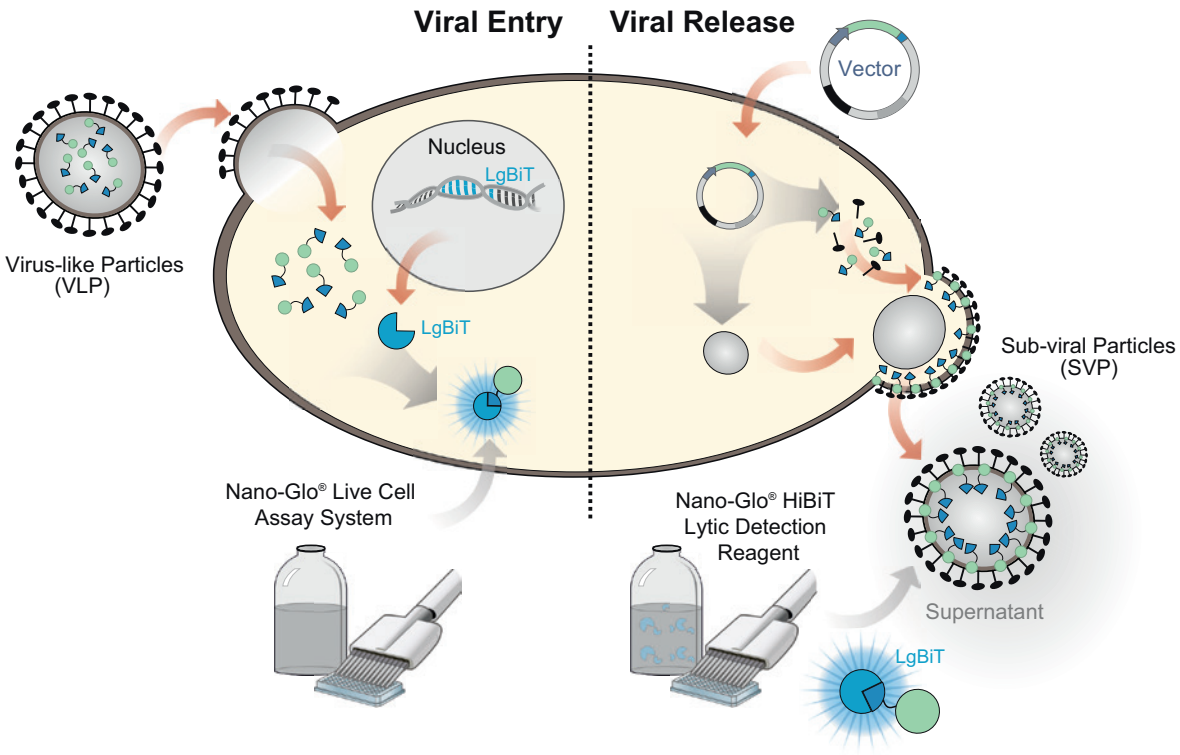
Rapid and antibody-free visualization of HIF1α stabilization from phenanthroline-treated cells using the *Nano-Glo® HiBiT Blotting System*. Cell lysates were blotted from transiently transfected cells with a PGK-promoter driven HiF1α-HiBiT expression construct.



Monitor Viral Infection and Viral Release

The small size of HiBiT facilitates its insertion into viral genomes, while complementation with LgBiT enables live-cell assays for viral entry and replication. Compared to traditional detection methods like ELISA, HiBiT offers simple HTS-compatible "add-and-read" assay formats with no antibodies or wash steps required.

Analysis of viral entry and release using HiBiT Protein Tagging System



Cellular infection by HiBiT-tagged Virus-like particles (VLP) can be monitored in live cells expressing the HiBiT-complementary LgBiT subunit by using the *Nano-Glo® Live Cell Assay System* (left). Furthermore, release of sub-viral particles (SVP) can be easily quantified by adding the SVP-containing supernatant to the *Nano-Glo® HiBiT Lytic Detection System* (right).

For representative examples of how HiBiT facilitated the development of rapid and quantitative assays for viral entry and release, see the following:

Miyakawa, K. *et al.* (2020) Rapid quantitative screening assay for SARS-CoV-2 neutralizing antibodies using HiBiT-tagged virus-like particles. *medRxiv* (preprint)

Yamamoto, M. *et al.* (2019) Cell-cell and virus-cell fusion assay-based analyses of alanine insertion mutants in the distal α9 portion of the JRFL gp41 subunit from HIV-1. *J Biol Chem.* **294(14):**5677–56787

Tamura, T. *et al.* (2019) *In vivo* dynamics of reporter Flaviviridae viruses. *J. Virol.* **93(22)**:e01191–19

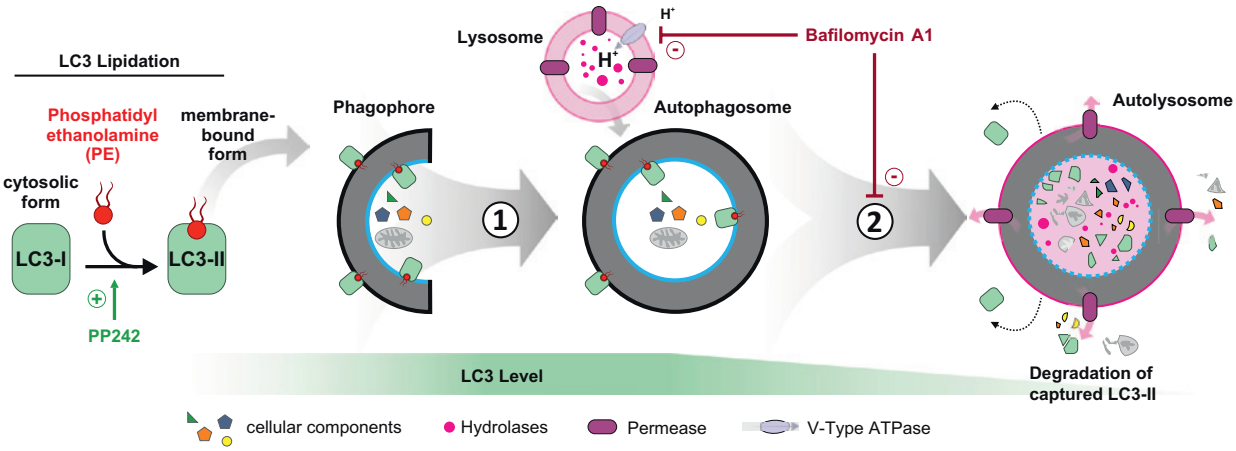
Sasaki, M. *et al.* (2018) Development of a rapid and quantitative method for the anlysis of viral entry and release using a Nanoluc luciferase complementation assay. *Virus Res.* **243**:69–74

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

Measure Autophagic Flux

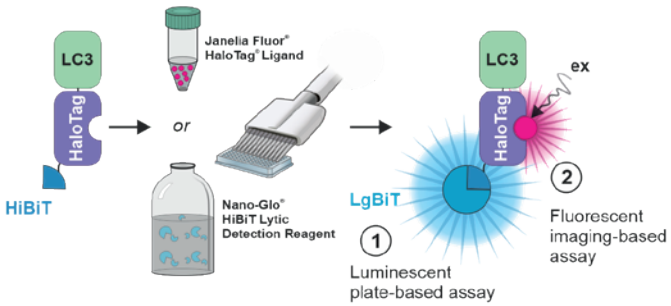
Autophagy is an important intracellular pathway for the degradation of superfluous or harmful subcellular materials, thereby playing a critical role in maintenance of cell health under normal and stress conditions. Changes in the total level of LC3 protein can be used to monitor changes in autophagic flux. HiBiT was used to generate an *Autophagy LC3 HiBiT Reporter Assay System* providing a homogeneous, bioluminescent, plate-based method for quantitative assessment of autophagy, including simple and reliable discrimination between inducers and inhibitors of the pathway.

Process of LC3 degradation during autophagy

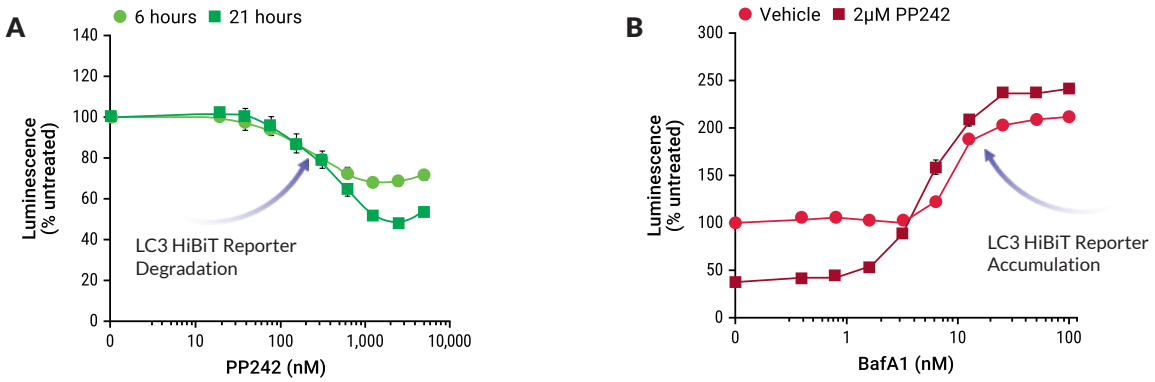


LC3 HiBiT Reporter – two assays in one

The *Autophagy LC3 HiBiT Reporter* has been engineered by tagging human LC3B with HiBiT and HaloTag®. The amount of LC3 HiBiT reporter is quantified in a lytic luminescent plate-based assay. The amount of luminescence is proportional to the amount of reporter in the lysate, so the assay signal is inversely correlated with the autophagic flux. Additionally, the bright *Janelia Fluor® HaloTag® Ligands* enable fluorescent imaging of changes in LC3 reporter localization.



Measuring autophagic flux with the Autophagy LC3 HiBiT Reporter Assay System



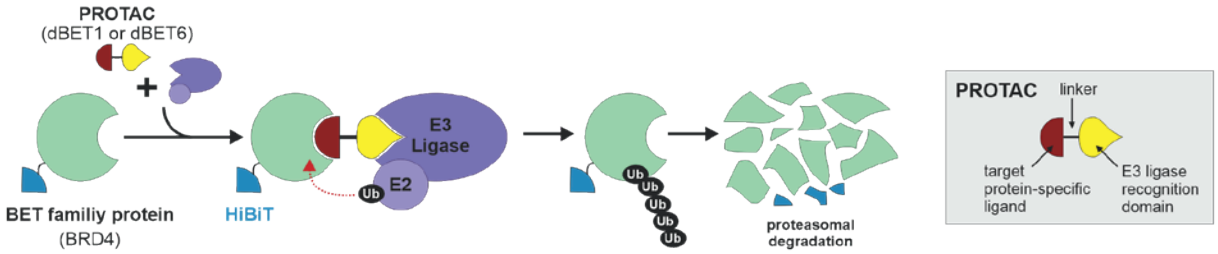
(A) Treatment of stable *U2OS Autophagy LC3 HiBiT Reporter Cells* with the autophagy-stimulating compound PP242 leads to a time- and concentration-dependent attenuation of the luminescence signal due to the degradation of the HiBiT-tagged autophagy reporter. **(B)** Reporter cells were treated with increasing concentrations of a reference inhibitor, Bafilomycin A1 (Baf A1), without (vehicle) or with 2 µM PP242 for 21 hours. Co-treatment with PP242 significantly increases the assay window for autophagy inhibitor detection.

Monitor Protein Degradation in Cells

The degradation of proteins is a common mechanism to alter the activities of signaling pathways. Besides knocking down a certain protein by RNAi or chemically inhibiting its molecular activity, the targeted degradation of proteins by so-called proteolysis-targeting chimeras (PROTACs) has become a promising strategy to pharmacologically interfere with cellular signaling, especially in cases of proteins lacking regulatory or active sites to bind traditional enzyme inhibitors.

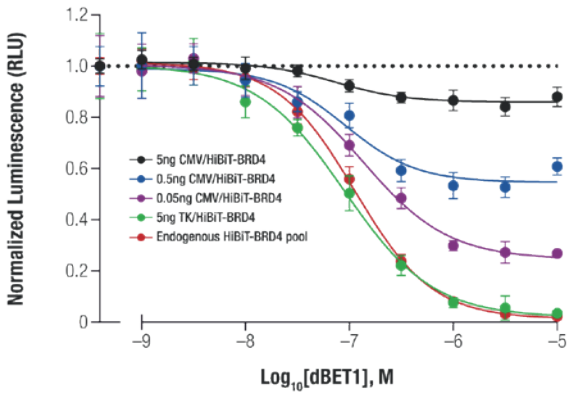
PROTACs – Targeting Proteins for Degradation

PROTACs are bifunctional molecules composed of a moiety that binds to the protein of interest coupled to a moiety that binds to a member of an E3 ligase complex. Simultaneous binding of each moiety brings the E3 ligase complex into proximity to the target protein, leading to its ubiquitination and subsequent proteasomal degradation. The PROTACs dBET1 and dBET6 were designed to direct the BET family proteins, e.g. bromodomain-containing protein 4 (BRD4), to the proteasome. By tagging the target protein with HiBiT, this process can be easily monitored.



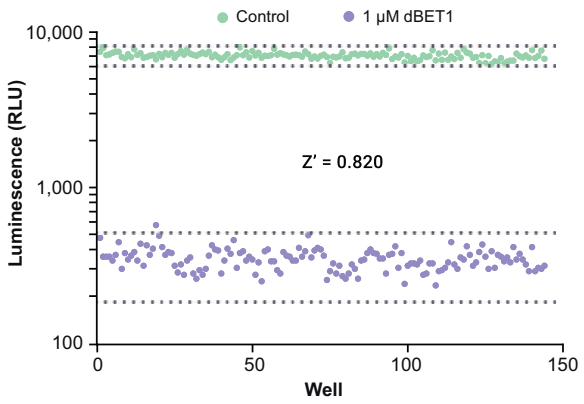
Monitoring targeted degradation of HiBiT-BRD4 in cells treated with the PROTAC dBET1

The magnitude of dBET1-dependent loss of HiBiT-BRD4 is influenced by its expression level. Overexpression likely overwhelms the endogenous ubiquitin ligases and proteasomes. Reducing expression levels by reducing the amount of transfected CMV expression vector, using a weaker TK promoter, or tagging the endogenously expressed protein improves the magnitude of the response. The kinetics of protein degradation (see next page) can only be studied using the endogenous protein, however, as the mechanisms regulating native protein vs ectopic protein turnover will influence degradation rate, extent, and recovery.



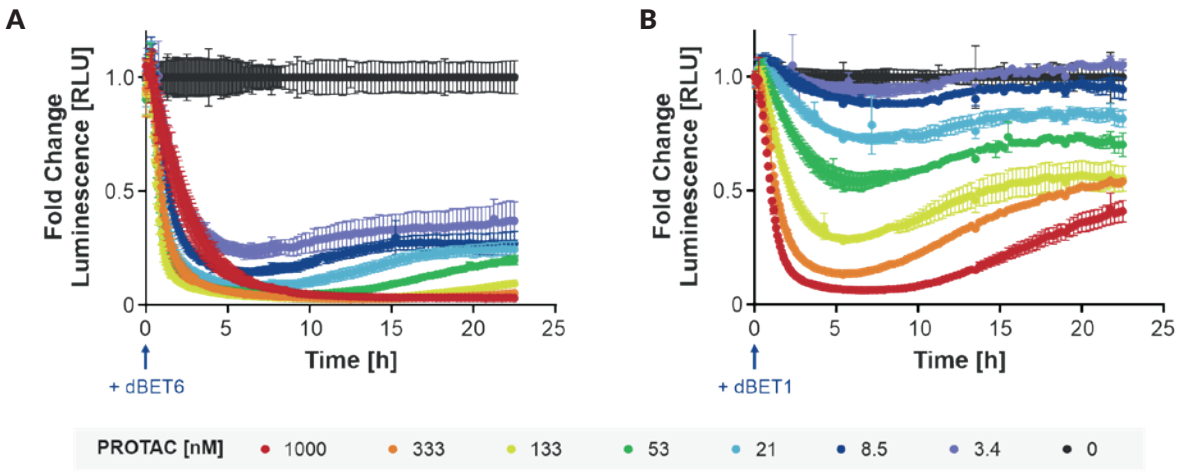
Induced degradation of HiBiT-BRD4 in a CRISPR-derived pool of cells in 384-well plates

The simple protocol and luminescence half-life of greater than 3 hours make the *Nano-Glo® HiBiT Lytic Detection System* ideal for batch processing multiple plates in high-throughput applications. The sensitivity and simplicity of HiBiT make it much more conducive to HTS assays of protein degradation than antibody-based methods.



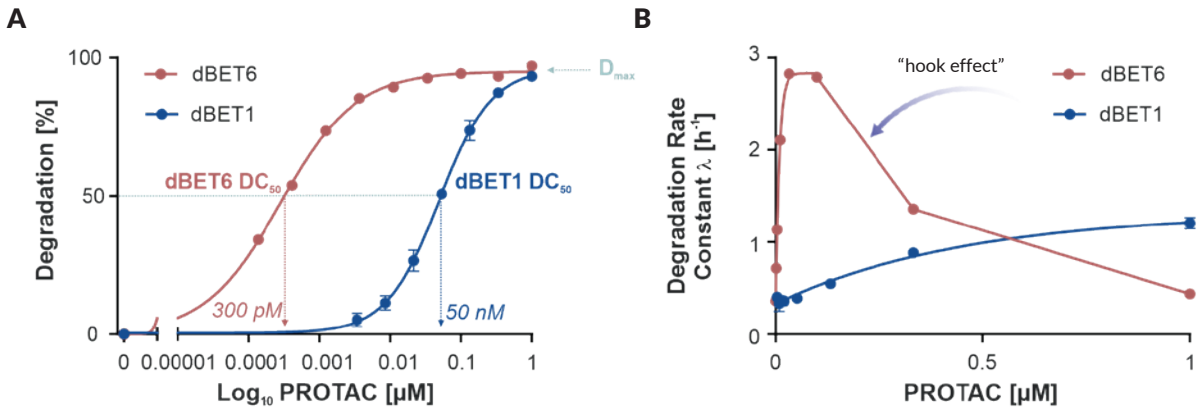
A New Way to Look at Protein Degradation

Quantification of protein loss in real-time upon PROTAC treatment



CRISPR-mediated tagging of BET family member BRD4 with HiBiT in cells stably expressing the LgBiT subunit enables the monitoring of targeted endogenous protein degradation in real-time. Before addition of PROTACs dBET6 **(A)** and dBET1 **(B)** at time zero, cells were pre-equilibrated with the extended *Nano-Glo® Endurazine™ Live-Cell Substrate*. The luminescent signal was recorded over a period of 24 hours to determine HiBiT-BRD4 degradation and recovery.

Calculation of quantitative parameters from real-time degradation profiles



Recording of real-time protein degradation and recovery profiles allows for determination of quantitative degradation parameters, i.e. percent degradation, half-maximal degradation concentration (DC_{50}), maximal level of degradation (D_{max}) **(A)**, and degradation rate **(B)**. These can be used for rank ordering of compounds. At high dBET6 concentrations, the degradation rate decreases due to hindered formation of ternary complexes (target protein:PROTAC:E3 ligase) also known as "hook effect".

Learn more about Promega technologies for studying (Targeted) Protein Degradation

Please visit
www.promega.com/protein-degradation

check for

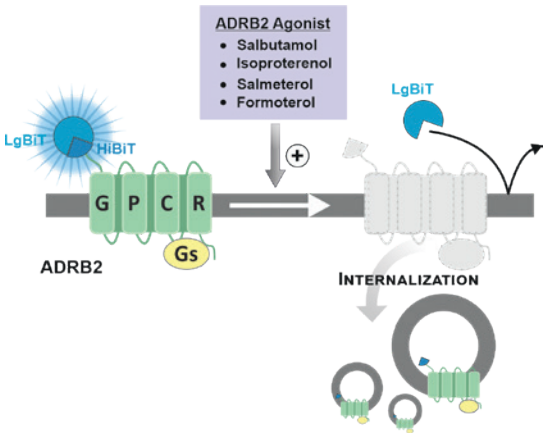
- PROTAC permeability
- Binary & ternary complex formation
- Ubiquitination
- Proteasomal recruitment

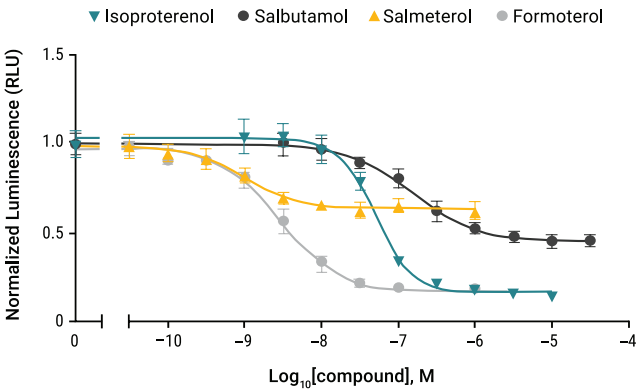
Surface Receptor Quantification & Internalization

The *Nano-Glo® HiBiT Extracellular Detection System* enables the development of simple, quantitative assays for receptor internalization that save time and eliminate the variability associated with antibody-based methods. Using the optimized detection reagent results in rapid equilibration with surface receptors to capture rapidly changing biology and minimize well-to-well variability.

Measure ligand potency and extent of GPCR receptor internalization in a few minutes

Stimulation of the β_2 adrenergic receptor (ADRB2) with different agonists leads to its internalization. When expressed as an externally tagged HiBiT fusion protein this process can be easily quantified using the *Nano-Glo® HiBiT Extracellular Detection System*, because LgBiT Protein is cell-impermeable and will only bind to surface receptors.



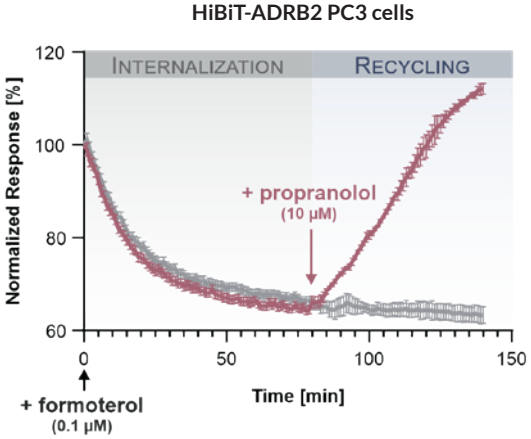


Agonist	EC_{50}	Percentage Receptor Remaining on Surface
Isoproterenol	50.9 nM	16 %
Salbutamol	161 nM	45 %
Salmeterol	1.04 nM	63 %
Formoterol	2.92 nM	16 %

The potency (EC_{50}) of different ADRB2 receptor agonists was determined in a homogenous plate-based assay and the degree of receptor internalization was calculated respectively. The known agonists of ADRB2 promoted receptor internalization with the expected differences in rank order potency. Additionally, the partial agonists salbutamol and salmeterol displayed the expected reduction in the extent of internalization.

Live-cell monitoring of receptor trafficking in real-time

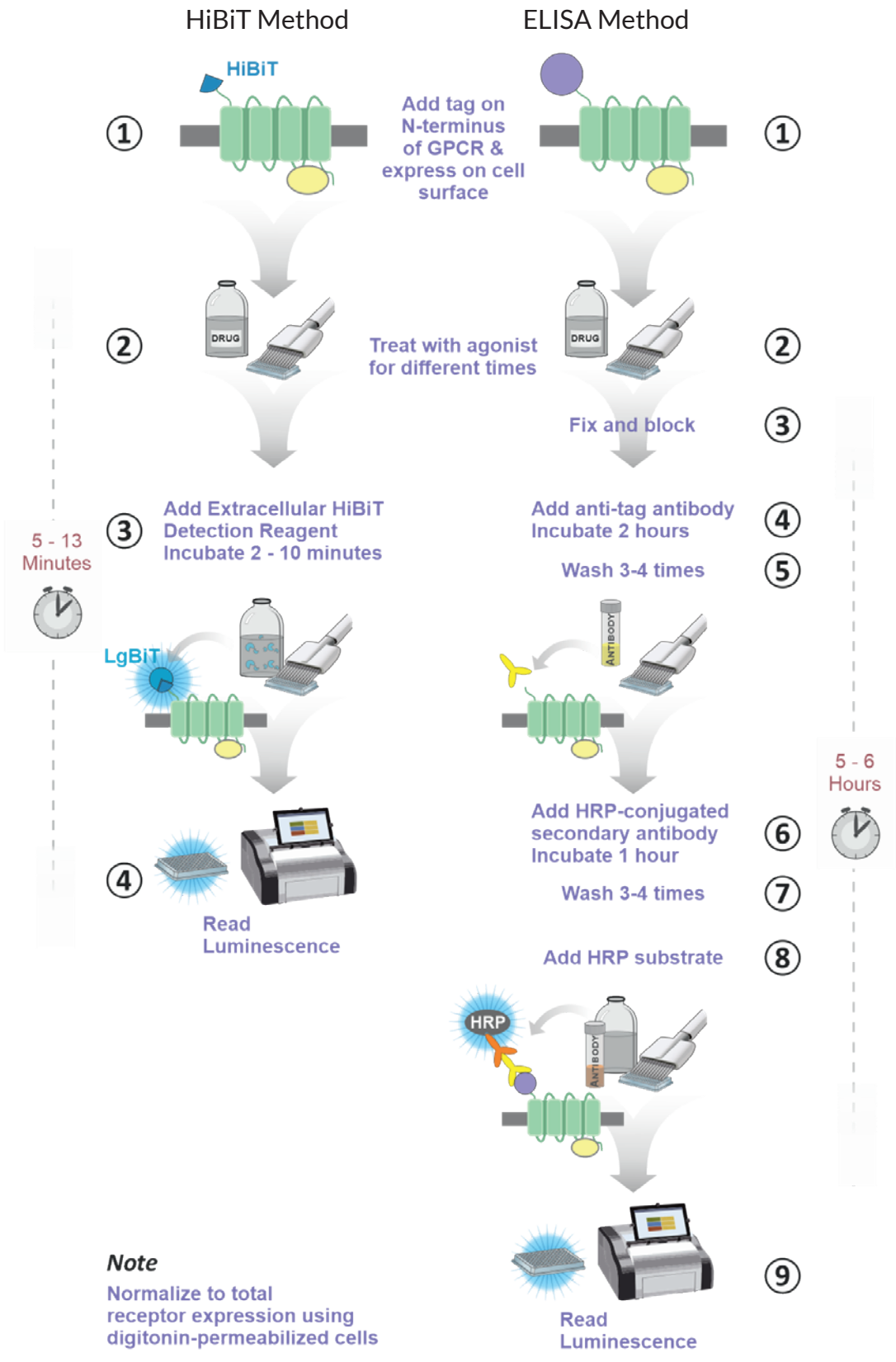
Addition of purified LgBiT Protein plus live-cell substrate (or extended substrate) enables real-time kinetic analysis of receptor trafficking even at endogenous expression levels. After pre-incubation of cells with LgBiT and substrate, the agonist formoterol induces ADRB2 receptor internalization. This results in a reduction in luminescence, presumably due to the effect of the lower endosomal pH or substrate availability on NanoBiT® luciferase activity. Endocytosis is stopped by addition of the antagonist propranolol, leading to gradual recycling of the receptor back to the surface as seen by a gain in signal (**red**), while it remains low without propranolol treatment (**grey**).



A Picture Paints a Thousand Words...

"We have done ELISA and now we only use HiBiT."
– Researcher from the University of Iowa –

The *HiBiT Protein Tagging System* offers a much faster, more sensitive, and less variable way to monitor receptor trafficking to and from the cell surface, compared to the labor-intensive ELISA method.

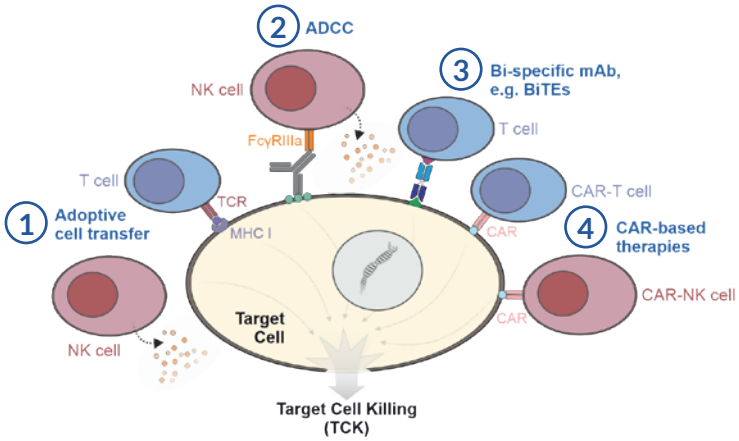


Determine Target Cell Killing in Co-Cultures

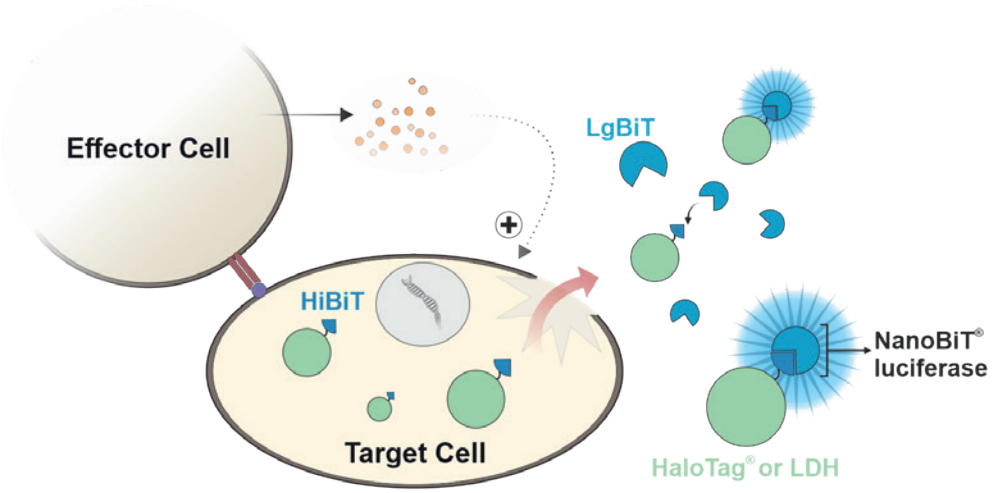
Target cell killing

A promising new strategy to fight cancer is to harness the body's own immune system. Various approaches exist that are based on directing specific immune effector cells to kill the cancer target cells. Research in this area therefore requires technologies to specifically detect death of a target cell population in a co-culture setting. Current methods either lack specificity (e.g. LDH release assay) or require radioactive labeling (e.g. ^{51}Chromium-release cytotoxicity assay), specialized instruments or lengthy workflow procedures. Among the various therapeutic strategies that have been developed to date are:

1. Adoptive T and NK cell transfer
2. Antibody-dependent cellular cytotoxiciy (ADCC)
3. Bi-specific monoclonal antibodies, e.g. bispecific T cell engager (BiTE)
4. Chimeric antigen receptor-based therapies, e.g. CAR-T and CAR-NK cells



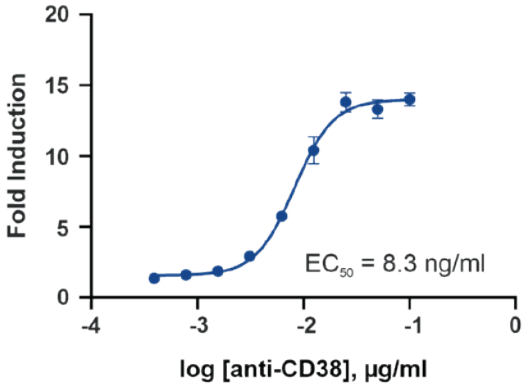
Principle of measuring target cell killing in co-cultures using HiBiT



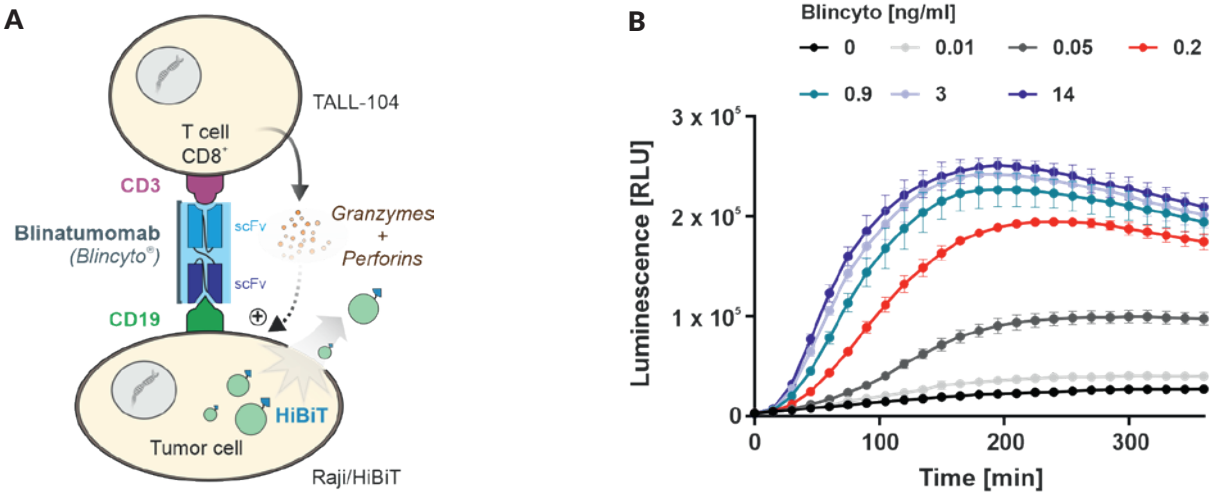
The *HiBiT Target Cell Killing Assay* is based on the release of a HiBiT fusion protein from target cells upon loss of membrane integrity. This event can readily be detected by addition of cell-impermeable LgBiT protein that immediately binds to extracellular HiBiT yielding a bioluminescent signal from the reconstituted HiBiT:LgBiT luciferase. The signal is proportional to the amount of target cell death. Target cells are engineered to ectopically express HaloTag®-HiBiT, whereby the HaloTag® fusion enables fluorescent imaging and positive clone isolation by FACS using the fluorescent *Janelia Fluor® 646 HaloTag® Ligand*. Alternatively, CRISPR technology can be used to add HiBiT to the endogenous locus of the commonly used cytosolic protein marker of cell death, lactate dehydrogenase (LDH).

Antibody-Dependent Cell-mediated Cytotoxicity (ADCC)

A549 cells (2,500 cells/well) stably expressing a HaloTag®-HiBiT fusion protein were incubated with primary PBMCs at an effector:target cell ratio of 20:1 in the presence of various concentrations of the therapeutic antibody Cetuximab for 5 hours. Target cell killing through ADCC was determined by using *Nano-Glo® HiBiT Extracellular Detection System*.



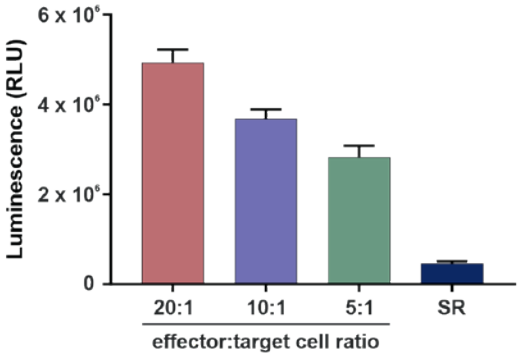
Redirected T Cell Cytotoxicity



(A) Bi-specific T cell engagers (BiTEs) resemble a fusion of two single-chain variable fragments (scFv) of two different monoclonal antibodies. They redirect T cell cytotoxicity to tumor cells by simultaneous binding of a T cell- and a tumor-specific antigen. Blincyto®, for example, binds to CD3 on T cells and CD19 on cancer target cells. The use of target cells stably expressing the HaloTag®-HiBiT fusion protein enables selective measurement of Blincyto®-induced target cell killing in the presence of activated TALL-104 CD8+ effector T cells. **(B)** This process can be monitored in real-time by addition of the *Nano-Glo® Endurazine™ Live Cell Substrate* in combination with cell-impermeable LgBiT protein.

CAR-T Cell Therapy

CAR-T cell mediated killing of HaloTag®-HiBiT expressing CD19+ K562 target cells was determined in an endpoint assay using *Nano-Glo® HiBiT Extracellular Detection System*. Target cells (2,500 cells/well) were incubated for 24 hours with anti-CD19 CAR-T cells (ProMab; PM-CAR1003) at different effector:target cell ratios. Background luminescence caused by spontaneous release (SR) was determined in the absence of effector cells.



Product Order Information

Nano-Glo® HiBiT Lytic Detection System

- Detection of HiBiT-tagged proteins in cell lysates
- Rapid & sensitive detection

Cat. #	Quantity
N3030	10 ml
N3040	100 ml
N3050	10 x 100 ml

Nano-Glo® HiBiT Extracellular Detection System

- Detection of HiBiT-tagged surface receptors or secreted proteins
- Monitor the dynamic of receptor internalization within minutes
- Antibody-free homogenous assay format

Cat. #	Quantity
N2420	10 ml
N2421	100 ml
N2422	10 x 100 ml

Nano-Glo® HiBiT Blotting System

- Detection of HiBiT-tagged proteins on Western blot membranes within minutes
- Antibody-free homogenous assay format

Cat. #	Quantity
N2410	100 ml

HiBiT Control Protein

- Purified recombinant HaloTag® protein (36 kDa) fused at its C-terminus to HiBiT (1.3 kDa)
- Use as a positive control of known concentration when using the Nano-Glo® HiBiT Lytic Detection System, Nano-Glo® HiBiT Extracellular Detection System or Nano-Glo® HiBiT Blotting System
- Detection of LgBiT and LgBiT fusion proteins

Cat. #	Quantity
N3010	100 µl (20 µM)

Co-Expression of LgBiT Subunit

	Cat. #	Quantity
LgBiT Expression Vector [CMV / Hygro]	N2681	20 µg
LgBiT-LentiB3 Transfer Vector	CS1956B33	20 µg
HaloTag®-LgBiT Expression Vector [CMV / Hygro]	CS1956B02	20 µg
HEK293 LgBiT Cell Line (stable)	N2672	2 vials
HeLa LgBiT Cell Line (stable)	CS1956D05	2 vials
Jurkat LgBiT Cell Line (stable)	CS1956D07	2 vials

Further cell lines available, please inquire

Nano-Glo® Live Cell Assay System

- Use to detect NanoBiT® protein complementation or NanoLuc® reporter activity
- Monitors luminescence at a single time point or continuously for up to **2 hours** without compromising cell viability

Cat. #	Quantity
N2011	100 assays
N2012	1000 assays
N2013	10,000 assays

Nano-Glo® Extended Live Cell Substrates

- Real-time quantification of HiBiT-tagged proteins
- Pro-Furimazine substrates whose slow cellular hydrolysis by esterases leads to steady release of furimazine throughout the experiment

Nano-Glo® Vivazine™ Substrate

- Ideal to support measurements from **2 to 24 hrs**

Cat. #	Quantity
N2580	0.1 ml
N2581	1 ml
N2582	10 ml

Nano-Glo® Endurazine™ Substrate

- Ideal to support measurements from **2 to 72 hrs**

Cat. #	Quantity
N2570	0.1 ml
N2571	1 ml
N2572	10 ml

Nano-Glo® Extended Live Cell Substrates Trial Pack

- Nano-Glo® Endurazine™ + Vivazine™ Substrate

Cat. #	Quantity
N2590	0.1 ml each

Need help with the design of your CRISPR/HiBiT tagging experiment?
Want to get a free recommendation for crRNA and donor DNA sequences?

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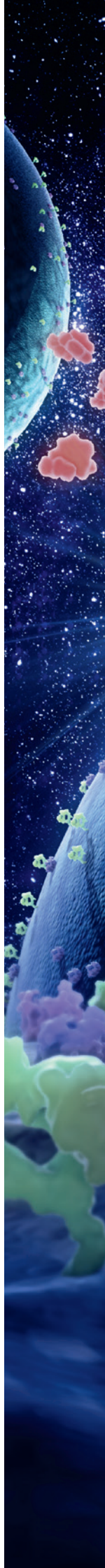
please contact our technical service



Product Order Information

HiBiT NanoDLR™

- Minimizing or eliminating experimental variability
- Control for specificity

	Cat. #	Quantity
Nano-Glo® HiBiT Dual-Luciferase® Reporter System	CS1956A08	10 ml
Nano-Glo® HiBiT Dual-Luciferase® Reporter System	CS1956A09	100 ml
pBiT4.1-C [HiBiT-IRES-luc2/CMV/Blast] Vector	CS1956B08	20 µg
pBit4.2-C [HiBiT-IRES-luc2/TK/Blast] Vector	CS1956B09	20 µg
pBiT4.3-C [HiBiT-IRES-luc2/PGK/Blast] Vector	CS1956B10	20 µg
pBiT4.1-N [HiBiT-IRES-luc2/CMV/Blast] Vector	CS1956B11	20 µg
pBit4.2-N [HiBiT-IRES-luc2/TK/Blast] Vector	CS1956B12	20 µg
pBiT4.3-N [HiBiT-IRES-luc2/PGK/Blast] Vector	CS1956B13	20 µg

HiBiT Fusion Vectors

- Choose the Flexi® vectors to enable rapid, convenient, and site-directed sub-cloning of inserts
- Choose the MCS vectors to alter linker length

	Cat. #	Quantity
pBiT3.1-N [CMV/HiBiT/Blast] Vector	N2361	20 µg
pBiT3.1-C [CMV/HiBiT/Blast] Vector	N2371	20 µg
pBiT3.1-secN [CMV/HiBiT/Blast] Vector	N2381	20 µg
pFN38K HiBiT CMV-neo Flexi® Vector	N2401	20 µg
pFC37K HiBiT CMV-neo Flexi® Vector	N2391	20 µg
pFN39K secHiBiT CMV-neo Flexi® Vector	N2411	20 µg

Autophagy LC3 HiBiT Reporter Assay System

	Cat. #	Quantity
U2OS Autophagy LC3 HiBiT Reporter Cell Line and Detection System	GA1050	1 kit *
HEK293 Autophagy LC3 HiBiT Reporter Cell Line and Detection System	GA1040	1 kit *
Autophagy LC3 HiBiT Reporter Vector and Detection System	GA2550	1 kit *
Janelia Fluor® 549 HaloTag® Ligand	GA1110	5 µg
	GA1111	3 x 5 µg
Janelia Fluor® 646 HaloTag® Ligand	GA1120	5 µg
	GA1121	3 x 5 µg

* 10 ml Nano-Glo® HiBiT Lytic Detection System included

HiBiT Target Cell Killing Assay

	Cat. #	Quantity
LgBiT Protein	CS1956A10	0.1 ml
	CS1956A11	1 ml
HaloTag®-HiBiT Vector [CAG / Blast]	CS1956B17	20 µg
HaloTag®-HiBiT Target Cells		
Raji Cells, Thaw-and-use	CS3055A02	1 vial
Raji Cells, Propagation Model	CS3055A04	2 vials
Ramos Cells, Thaw-and-use	CS3055A05	1 vial
Ramos Cells, Propagation Model	CS3055A07	2 vials
A549 Cells, Thaw-and-use	CS3055A08	1 vial
A549 Cells, Propagation Model	CS3055A10	2 vials
H929 Cells, Thaw-and-use	CS3055A11	1 vial
H929 Cells, Propagation Model	CS3055A13	2 vials
SK-BR-3 Cells, Thaw-and-use	CS3055A37	2 vials
SK-BR-3 Cells, Propagation Model	Please inquire	1 vial
K562 Cells, Thaw-and-use	Please inquire	2 vials
K562 Cells, Propagation Model	CS3000A06	1 vial
U937 Cells, Thaw-and-use	Please inquire	2 vials
U937 Cells, Propagation Model	CS3000A08	1 vial
LDH-HiBiT Target Cells		
Raji Cells, Propagation Model	CS3000A18	2 vials
Raji CD19-KO Cells, Propagation Model	CS3000A20	2 vials
Ramos Cells, Propagation Model	CS3000A22	2 vials
Ramos CD19-KO Cells, Propagation Model	CS3000A24	2 vials
OVCAR-3 Cells, Propagation Model	CS3000A29	2 vials
SC-OV-3 Cells, Propagation Model	CS3000A33	2 vials
A549 Cells, Propagation Model	Please inquire	2 vials
T2 Cells, Propagation Model	CS3000A35	2 vials
PBMC ADCC Bioassays		
PBMC ADCC Bioassay Kit (Raji)	CS3055A16	1 kit*
PBMC ADCC Bioassay Kit (Ramos)	CS3055A20	1 kit*
PBMC ADCC Bioassay Kit (A549)	CS3055A24	1 kit*
PBMC ADCC Bioassay Kit (H929)	CS3055A28	1 kit*
PBMC ADCC Bioassay Kit (SK-BR-3)	CS3055A36	1 kit*

Further cell lines available, please inquire

* cell culture medium, FBS and 10 ml Nano-Glo® HiBiT Extracellular Detection System included

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Detection of Bioluminescence

GloMax® Discover is an advanced multimode plate reader designed to provide optimal performance for Promega reagents with 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.

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- Multiplexing
- Assays for the detection of oxidative stress and cell metabolism
- ELISA
- BRET/FRET analysis

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