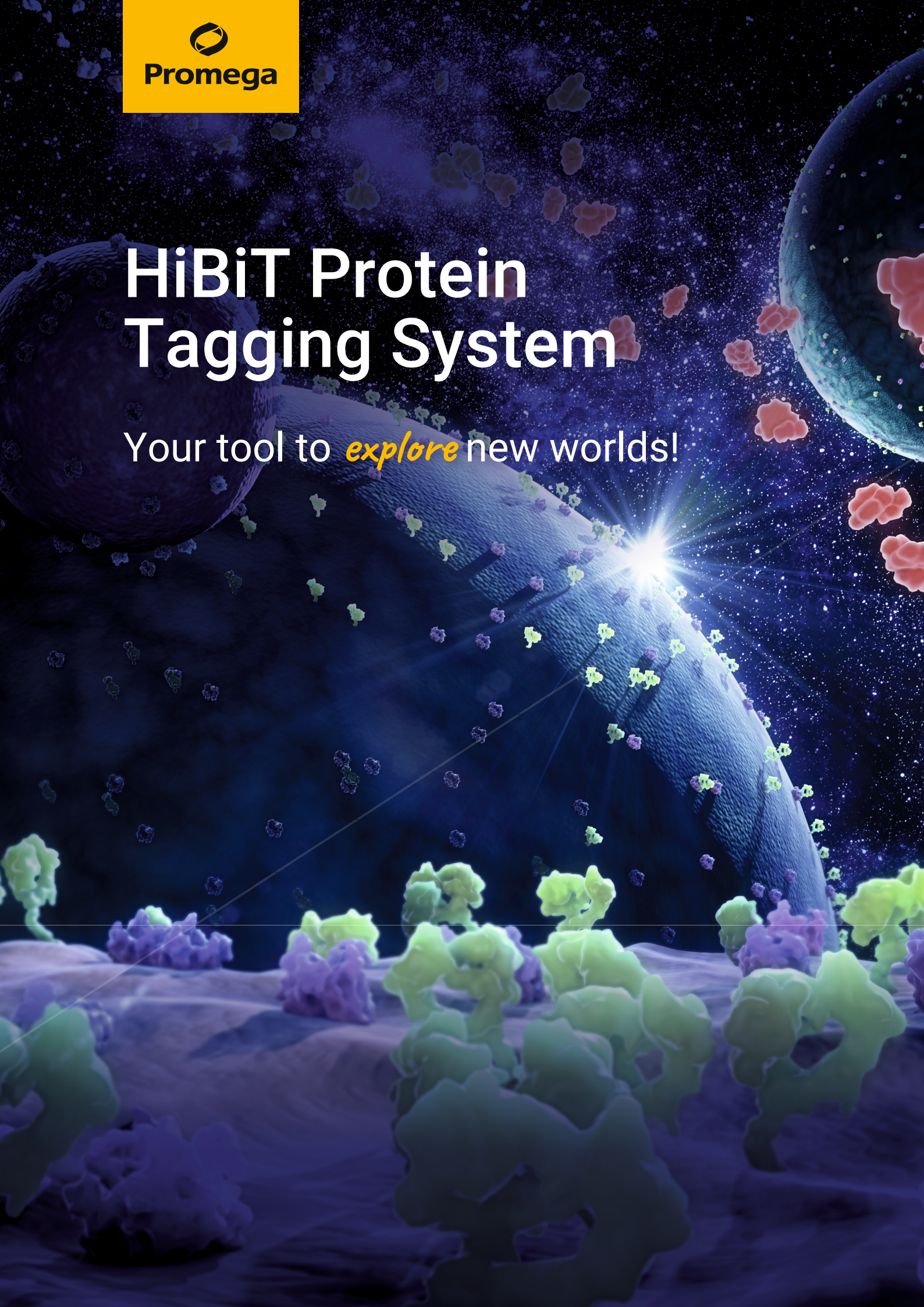
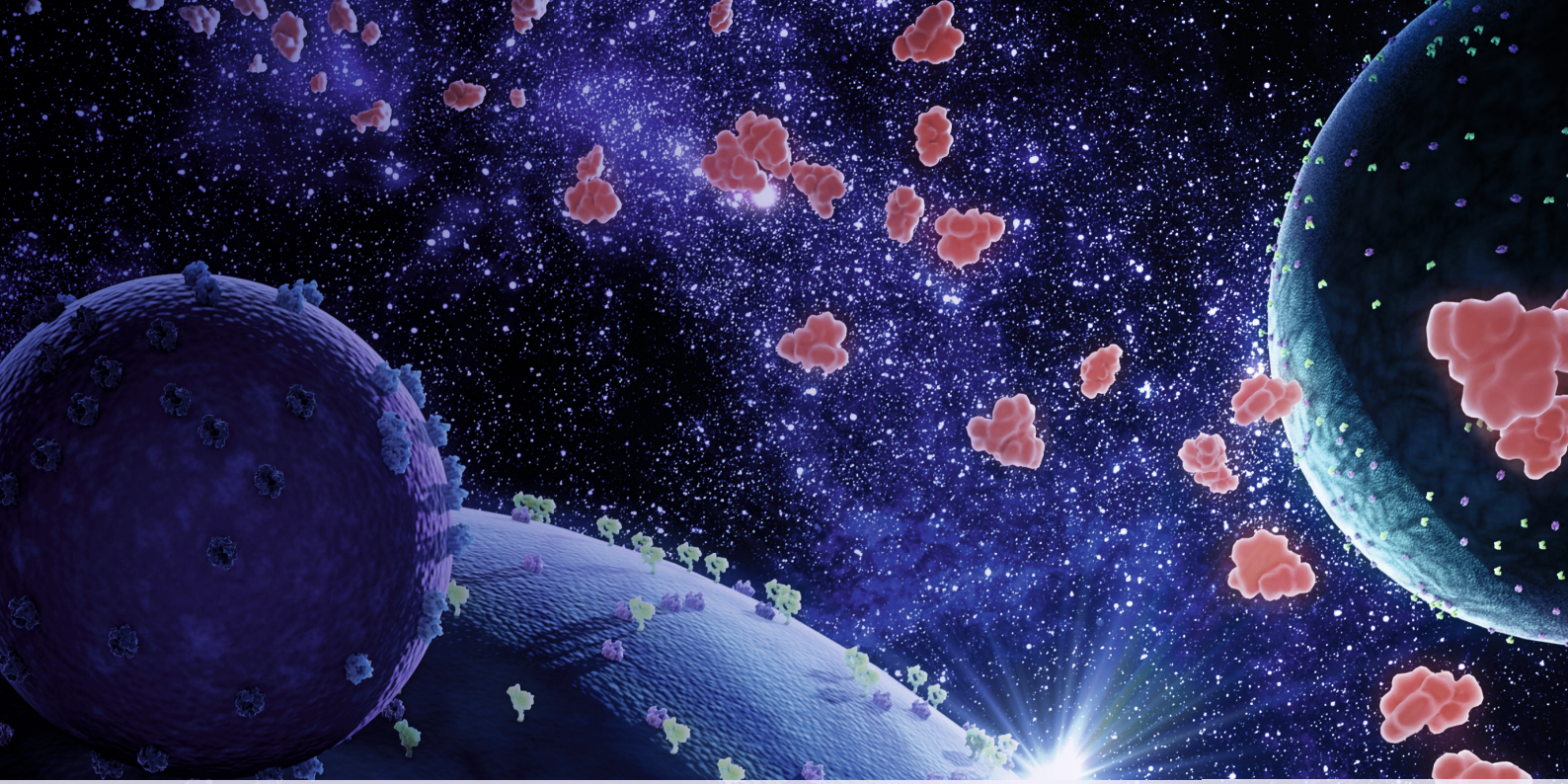
Promega

HiBiT Protein Tagging System

Your tool to *explore* new worlds!

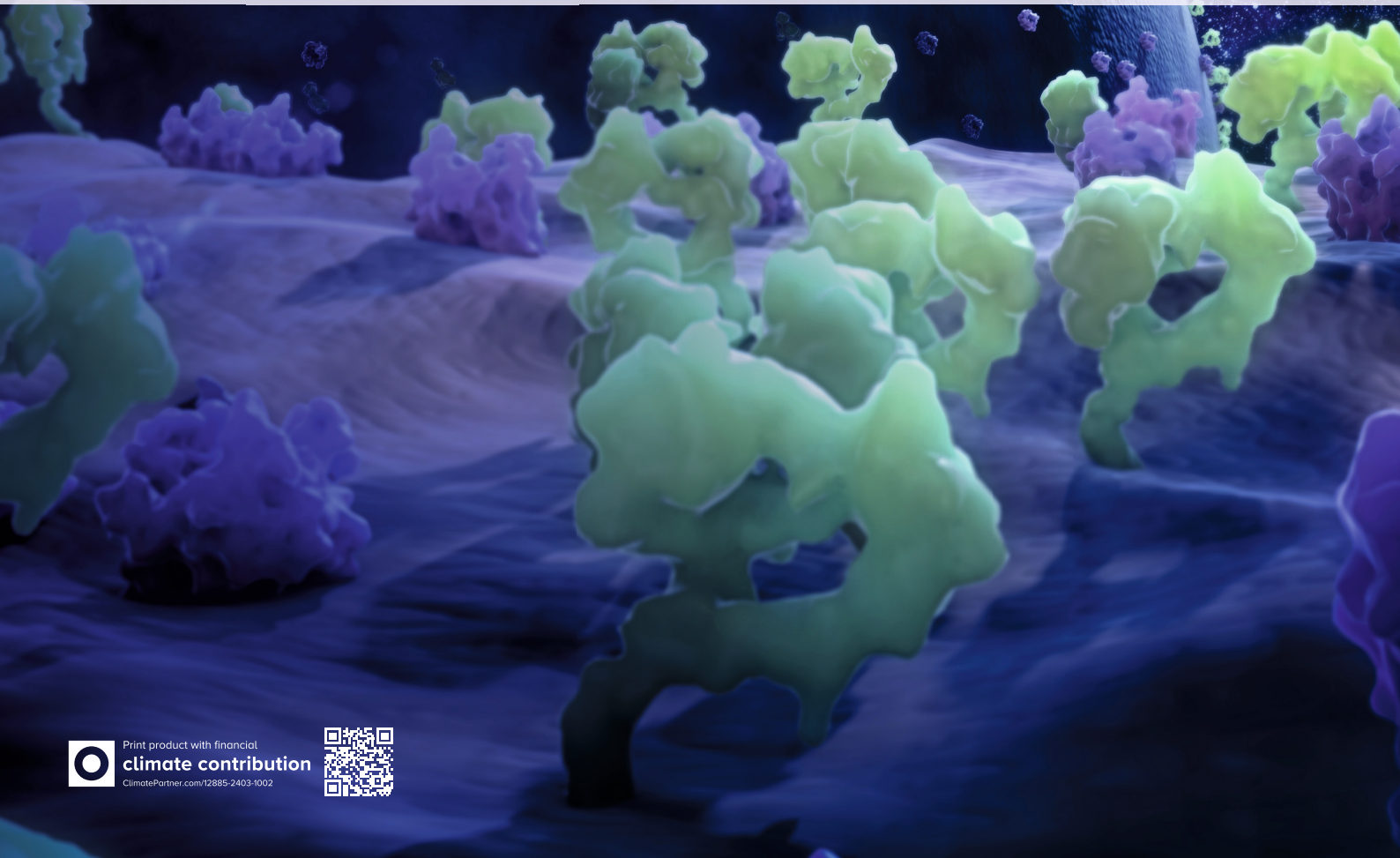




Your tool to *explore* new worlds!

Advanced Protein Quantification

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



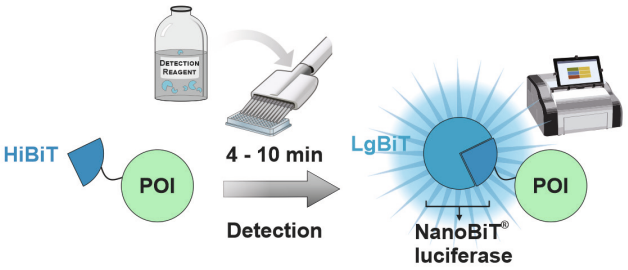
HiBiT Protein Tagging System

brings the power of bioluminescence to protein analysis

The *HiBiT Protein Tagging System* simplifies protein research, providing a simple and highly sensitive method for assessing changes in protein abundance and localization. This system eliminates the need for antibodies and enables live cell analysis of HiBiT-tagged proteins within minutes on a conventional luminometer. Due to its ability to detect endogenously expressed proteins and the convenient single reagent addition workflow, HiBiT technology unlocks a universe of possibilities for scientists in the field of protein biology.

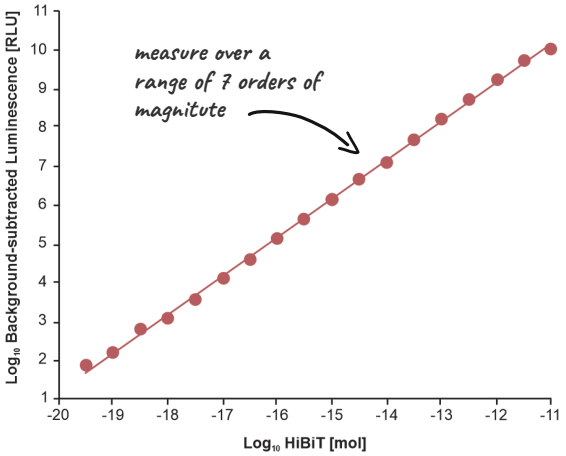
Principle

HiBiT (High BiT), an 11 amino acid peptide tag, can be appended to any protein-of-interest (POI). It enables detection in under 10 minutes using bioluminescent add-and-read reagents. These comprise an inactive luciferase subunit, LgBiT (Large BiT), which spontaneously associates with HiBiT to reconstitute active NanoBiT® luciferase.



Protein quantification with superior sensitivity

Featuring a wide dynamic range, the HiBiT Detection Systems accurately quantify HiBiT-tagged proteins across varying expression levels. With a detection limit of less than 10^{-19} moles even endogenously expressed proteins can be reliably detected.



Key publications

Na, L. *et al.* (2024) Avian ANP32A incorporated in avian influenza A virions promotes interspecies transmission by priming early viral replication in mammals. **Sci Adv. 10(5):eadj4163**

Ondra, M. *et al.* (2023) CRISPR/Cas9 bioluminescence-based assay for monitoring CFTR trafficking to the plasma membrane. **Life Sci Alliance. 7(1):e202302045**

Behbahanipour, M. (2023) OligoBinders: Bioengineered Soluble Amyloid-like Nanoparticles to Bind and Neutralize SARS-CoV-2. **ACS Appl Mater Interfaces. 15(9):11444-11457**

Garvin, D. *et al.* (2021) Determining ADCC activity of antibody-based therapeutic molecules using two bioluminescent reporter-based Bioassays. **Curr Protoc. 1(11):e296**

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**

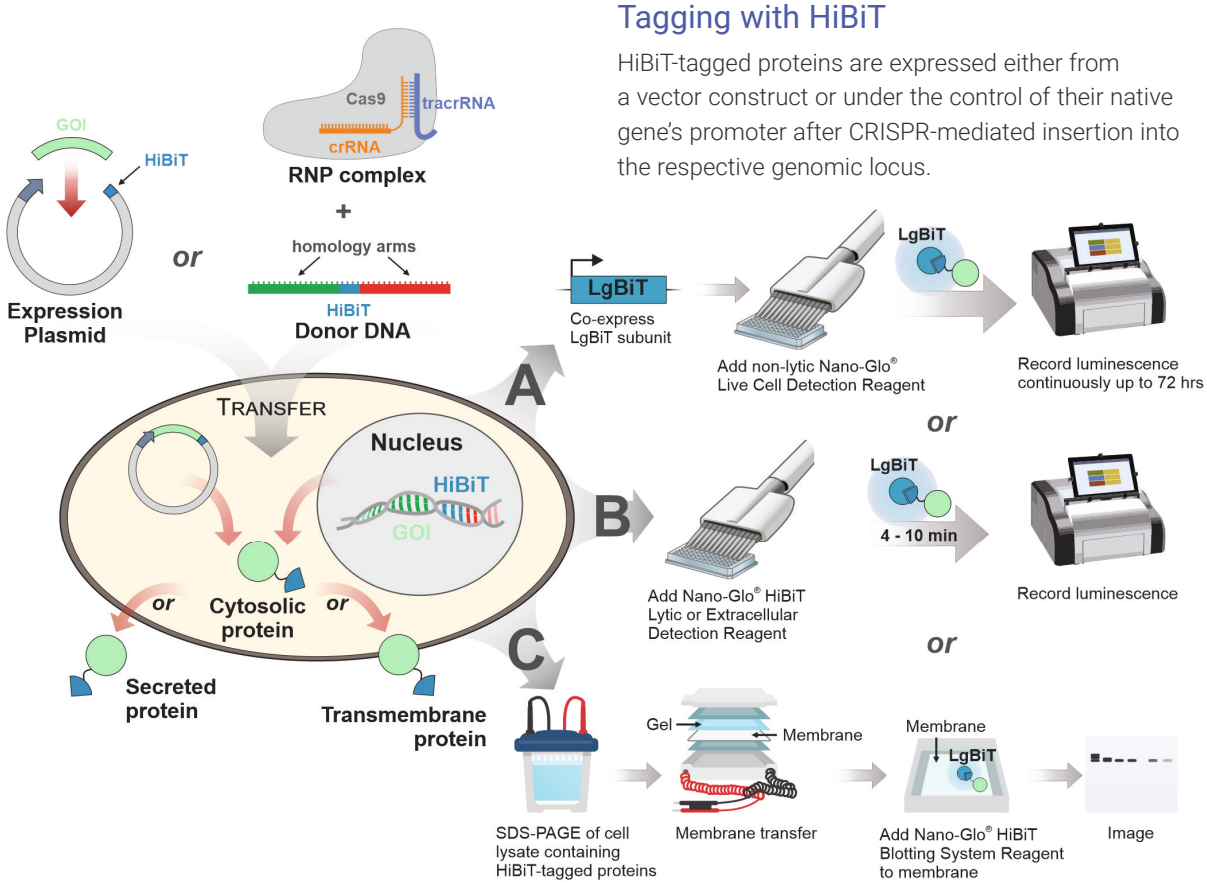
Sasaki, M. *et al.* (2018) Development of a rapid and quantitative method for the analysis of viral entry and release using a NanoLuc luciferase complementation assay. **Virus Res. 243:69–79**

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

HiBiT Tagging & Quantification Workflow

Tagging with HiBiT

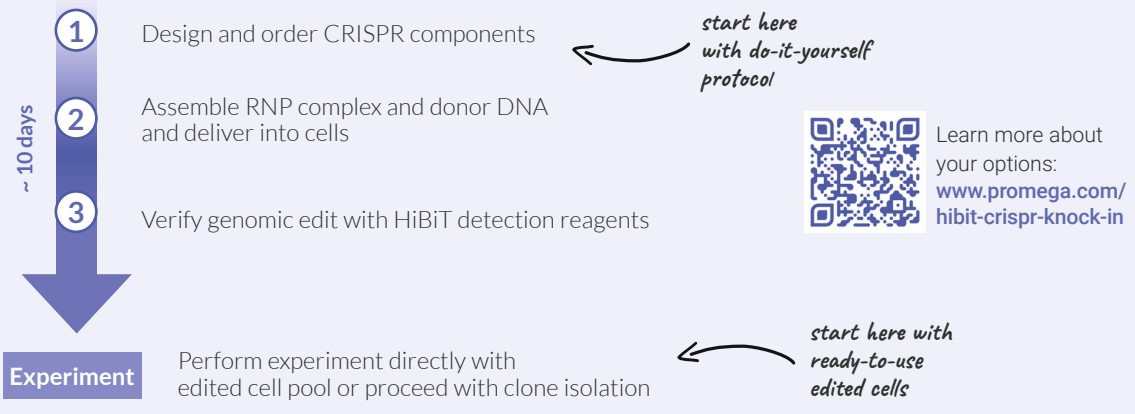
HiBiT-tagged proteins are expressed either from a vector construct or under the control of their native gene's promoter after CRISPR-mediated insertion into the respective genomic locus.



Quantification of HiBiT-tagged proteins: The amount of HiBiT-tagged proteins can be easily quantified using "add-and-read" assays without the need for 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 hours. **(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

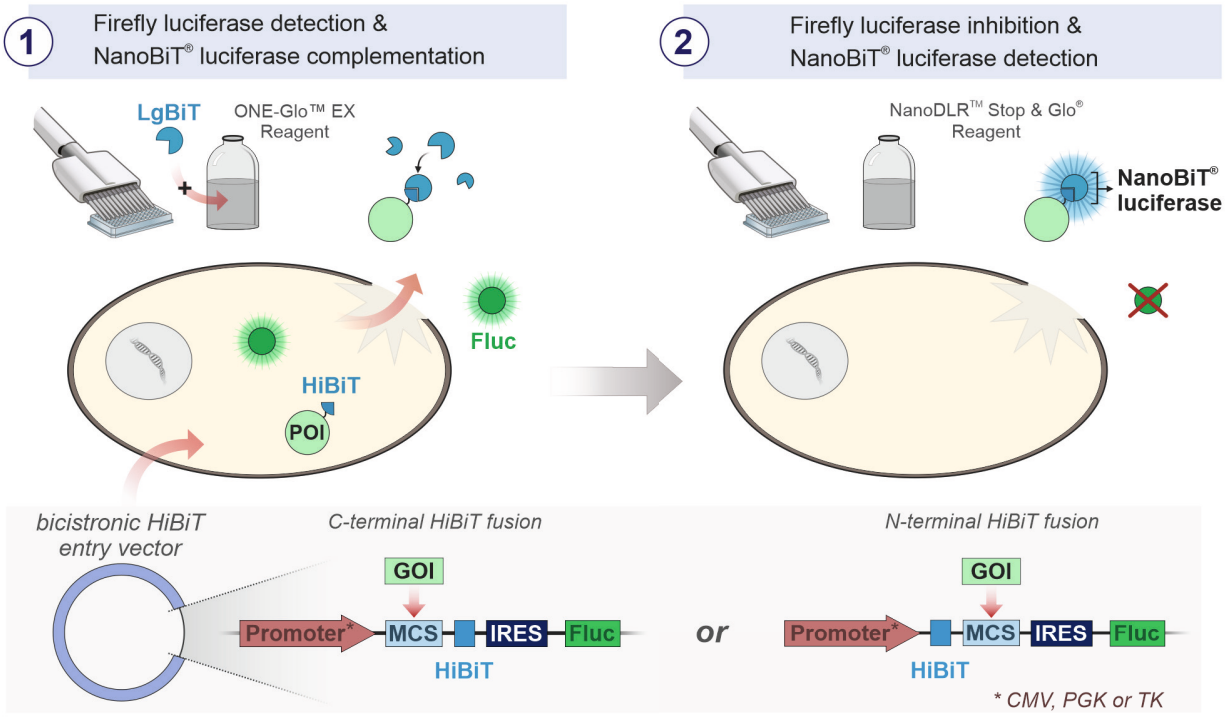
Either ***do-it-yourself*** with a cloning-free protocol for rapid success or choose from our growing collection of assay-ready CRISPR-edited cell lines for maximum convenience!



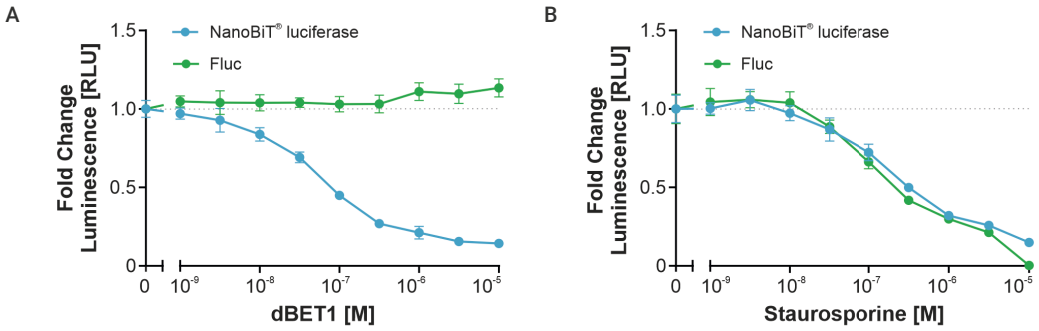
Specificity Control via Reporter Normalization

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.



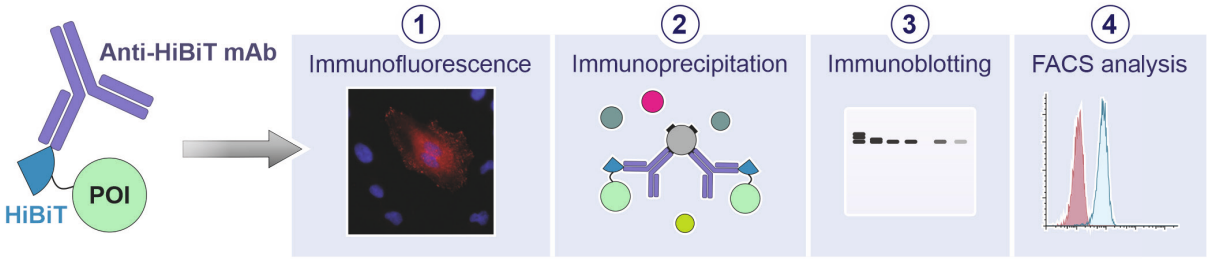
The coding sequence of the POI (GOI) is cloned into a multiple cloning site (MCS) with either an N- or C-terminal HiBiT fusion. Following the stop codon for the HiBiT fusion protein is an IRES sequence that enables constitutive co-expression of non-fused Fluc from the same mRNA. In the first step **(1)**, 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.



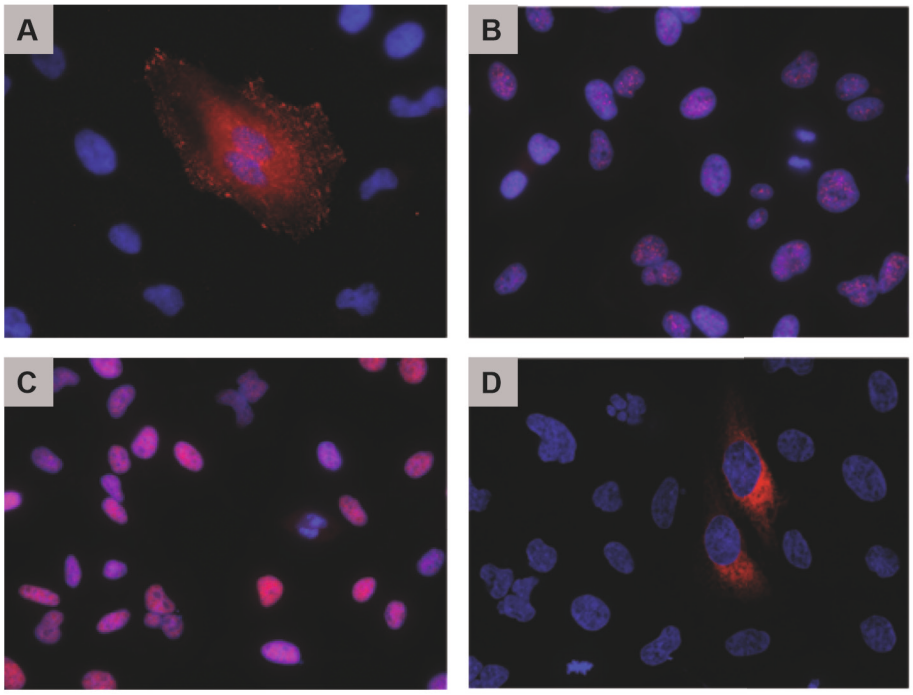
HEK293 cells were transiently transfected with a TK-driven bicistronic expression construct for HiBiT-BRD4 and Fluc. **(A)** Treatment of cells with dBET1, a PROTAC compound targeting BRD4 leads to specific degradation of HiBiT-BRD4 but not the Fluc control. **(B)** Treatment with the toxic compound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

Antibody-Based Methods for HiBiT Detection

The *Anti-HiBiT Monoclonal Antibody* is a mouse monoclonal antibody (mAb) that binds to the HiBiT tag with high affinity (KD ~ 6 pM) and specificity. It expands the detection options for HiBiT-tagged proteins, enabling traditional antibody-based applications such as immunoblotting, immunofluorescence, immunoprecipitation (IP) and fluorescence-activated cell sorting (FACS). Because of its high affinity and low cross-reactivity, many HiBiT-tagged proteins can be detected at endogenous expression levels.

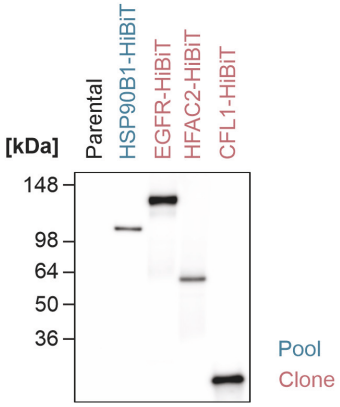


Immunofluorescence



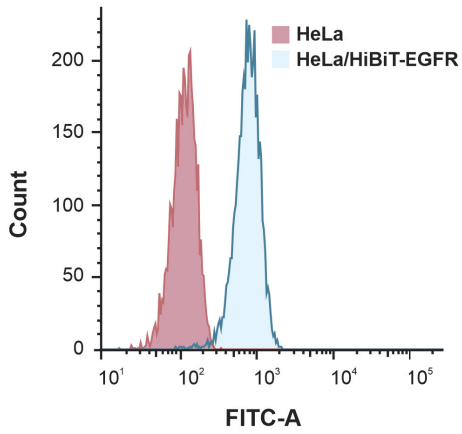
Immunofluorescent detection of HiBiT-tagged proteins in CRISPR-edited cell pools and clones using the Anti-HiBiT Monoclonal Antibody. Using CRISPR/Cas9 genome-editing technology in HeLa cells, the HiBiT tag was added to the C terminus of VCL (**Panel A**; focal adhesions, membrane, cytoskeleton), SMARCA4 (**Panel B**; nucleoli fibrillar centers), HDAC2 (**Panel C**; nucleus), and HSP90B1 (**Panel D**; endoplasmic reticulum, visualized with structured illumination). For HDAC2 and SMARCA4, HiBiT-positive clones were isolated. Fixed cells were stained with Anti-HiBiT Monoclonal Antibody and an Alexa Fluor® 647-labeled anti-mouse secondary antibody. The overlay with Hoechst nuclear stain (blue) is shown.

Immunoblotting



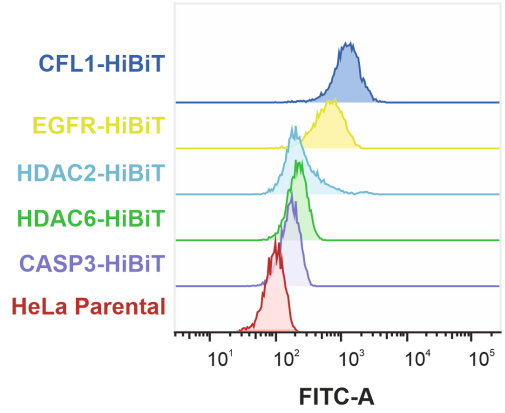
Lysates of CRISPR/HiBiT-edited HeLa cells were analyzed by immunoblotting using the Anti-HiBiT Monoclonal Antibody. The HiBiT-encoding sequence was added to the C-terminus of HSP90B1, EGFR, HDAC2 and CFL1 using CRISPR/Cas9 genome-editing technology. Cell lysates were generated from an edited cell pool predominantly containing HiBiT-negative cells (HSP90B1-HiBiT) or isolated HiBiT-positive cell clones (EGFR-HiBiT, HDAC2-HiBiT and CFL1-HiBiT). Unedited HeLa cells (parental) were used as negative control.

FACS analysis of live cells



Live-cell FACS analysis of a heterozygous HeLa/HiBiT-EGFR clonal cell line. Cells were stained with the Anti-HiBiT Monoclonal Antibody (1µg/ml) followed by a 1:100 dilution of the FITC anti-mouse IgG secondary antibody (BioLegend; #406001) A BSA containing FACS buffer was used to prevent receptor internalization. Unedited HeLa cells (parental) were used as negative control.

FFACS analysis of fixed cells

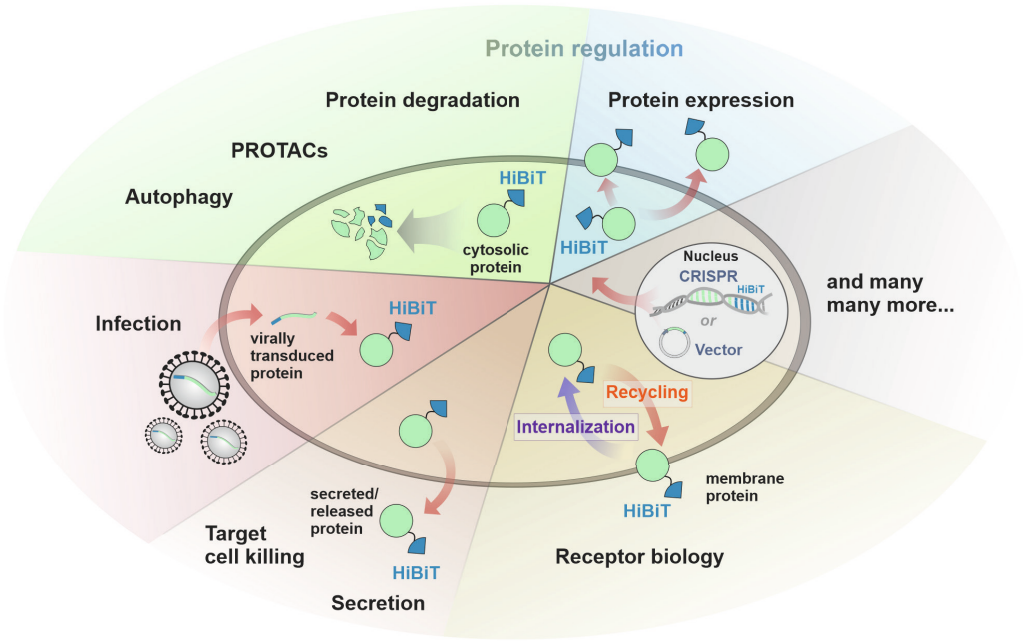


CRISPR-modified HeLa cells (CFL1-HiBiT; EGFR-HiBiT; HDAC2-HiBiT; HDAC6-HiBiT; CASP3-HiBiT) were fixed/permeabilized Cyto-Fast™ Fix/Perm Buffer Set (BioLegend; #426803). Upon staining with the Anti-HiBiT Monoclonal Antibody (1µg/ml) and an 1:100 dilution of the FITC anti-mouse IgG secondary antibody (BioLegend; #406001), cells were analyzed by FACS. Unedited HeLa cells (parental) were used as negative control.

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
- Antibody-based detection

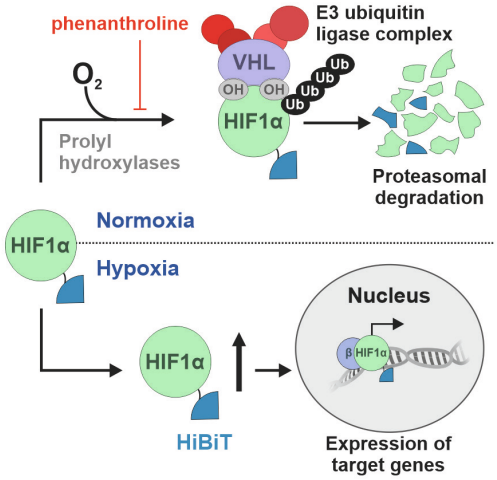
Endogeneous expression for highest response

Monitoring Post-Translational Regulation in Cells

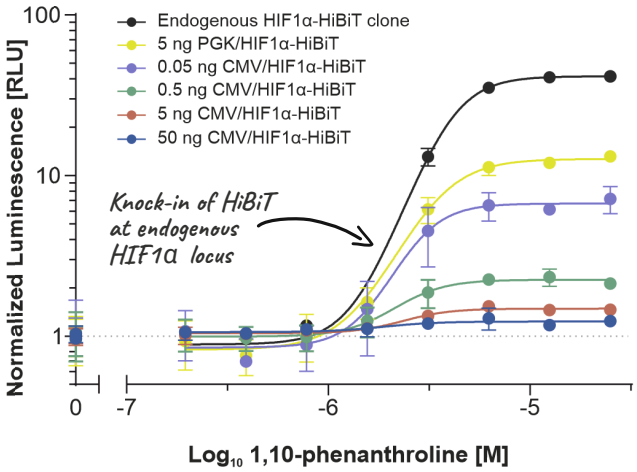
The HiBiT Tagging Technology can be easily applied to monitor post-translational regulatory processes such as ubiquitin-mediated degradation as shown below for the transcription factor HiF1α. For this experiment the *Nano-Glo® HiBiT Lytic Detection System* was used to measure regulated changes in protein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

Stabilization of transcription factor HIF1α

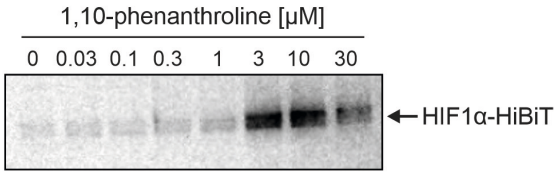
The level of the constitutively expressed transcription factor HIF1α is controlled by the presence of molecular oxygen (O_2). Under normoxic conditions HIF1α becomes prolyl hydroxylated in an oxygen-dependent reaction by prolyl hydroxylases. This modification leads to the recruitment of the von-Hippel-Lindau (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.

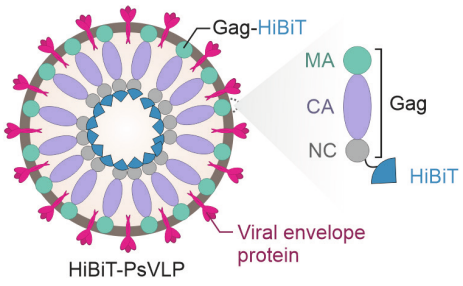


Rapid and antibody-free visualization of HIF1α stabilization from phenanthroline-treated cells using the *Nano-Glo® HiBiT Blotting System*. Cell lysates were blotted from transiently transfected cells with a PGK-promoter driven HiF1α-HiBiT expression construct.

Monitor Viral Infection & Neutralization

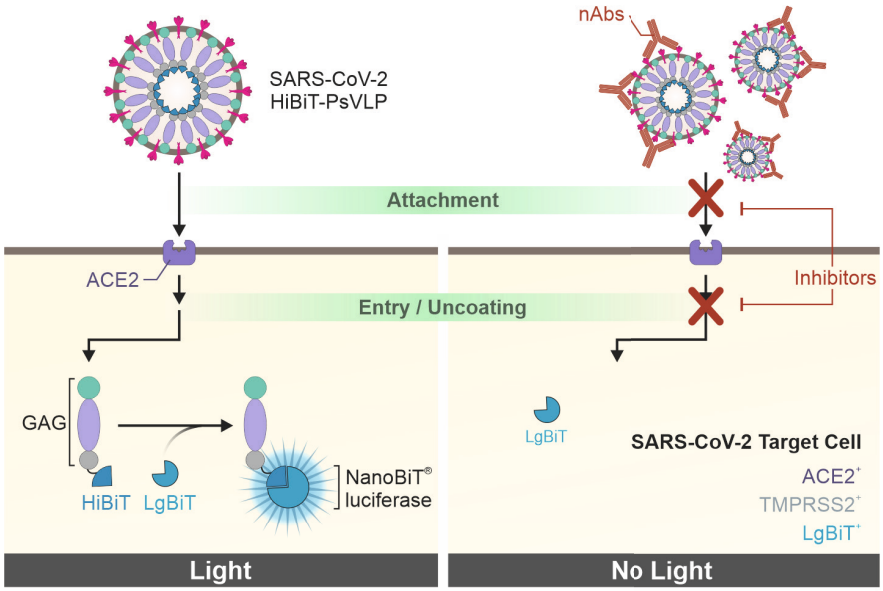
Luminescence technologies have been extensively applied in the study of viral biology. The compact size of HiBiT makes it particularly suitable for incorporation into small viral genomes and for translational fusion with viral proteins. The intense luminescent signal emitted by NanoBiT® luciferase enables highly sensitive detection of viral activity in both cells and organisms.

Study viral infection using HiBiT PsVLPs



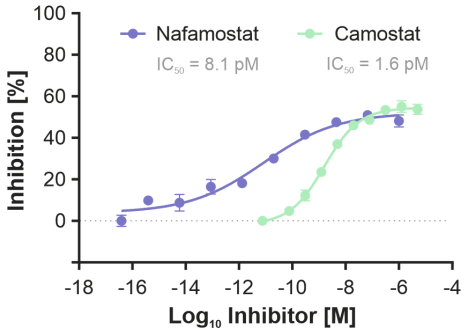
Virus-like particles (VLP) represent a powerful tool for studying viral infection, mitigating the need for higher biosafety infrastructure. The Gag polyprotein from HIV has been widely employed to generate these viral model systems. This precursor protein represents a translational fusion of three proteins, i.e. the matrix (MA), the capsid (CA) and the nucleocapsid (NC) protein. For the generation of HiBiT VLPs, a MA-CA-NC(Gag)-HiBiT fusion is expressed in HEK293 cells. By co-expression of a given viral envelop protein, HiBiT pseudotyped VLPs (HiBiT-PsVLP) can be generated to assess attachment and entry of a particular enveloped virus (e.g. SARS-CoV-2) of interest.

SARS-CoV-2 HiBiT-PsVLP Bioassay



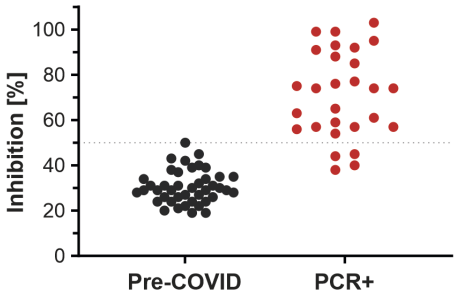
The SARS-CoV-2 HiBiT-PsVLP Bioassay is a bioluminescent reporter cell-based assay used to measure the neutralization capacity of ligands or antibodies that bind and block viral entry. This simple, convenient, and safe bioassay overcomes many limitations of the existing methods for determining neutralizing activity, e.g. plaque reduction neutralization test (PRNT) and surrogate viral neutralization tests (sVNT). This assay uses SARS-CoV-2 Target Cells that stably express the entry receptor ACE2, the surface protease TMPRSS2, as well as the complementary NanoBiT® luciferase subunit LgBiT. Upon infection of target cells by SARS-CoV-2 HiBiT-PsVLPs the functional NanoBiT® luciferase is rapidly reconstituted yielding a luminescent signal (left). Molecules that inhibit the attachment and entry/uncoating process are evident by causing a decrease in the luminescent signal relative to the non-treated control (right).

Determine potency of small molcule viral entry inhibitors



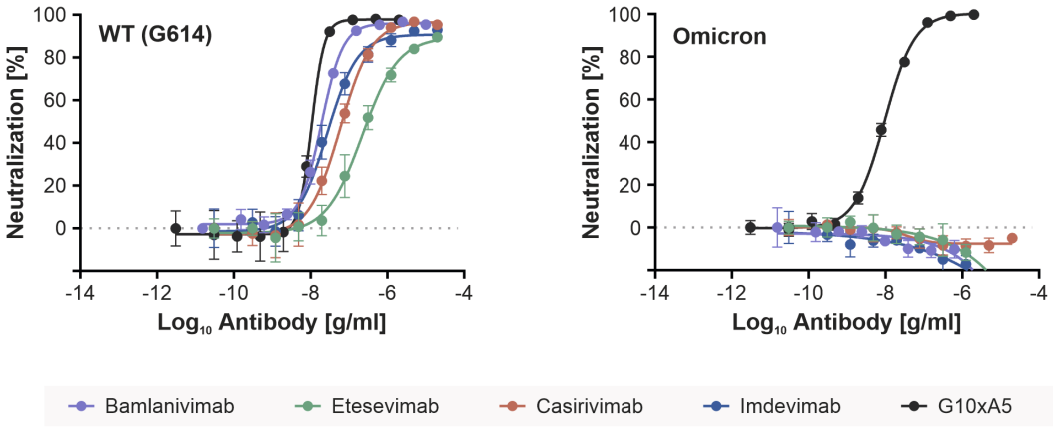
SARS-CoV-2 HEK293T Target Cells were incubated for 30 minutes with two small-molecule TMPRSS2 protease inhibitors. Upon addition of SARS-CoV-2 S(G614) HiBiT-PsVLPs and incubation for 3 hours, luminescence was determined using the *Nano-Glo® Live Cell Assay System*. Nafamostat inhibits SARS-CoV-2 entry with higher potency than camostat, consistent with published reports.

Screen human sera for SARS-CoV-2 viral neutralization



Human sera were analyzed for neutralization of SARS-CoV-2 S(G614). In comparison to pre-pandemic sera (Pre-COVID; n = 42), collected sera from individuals who tested positive for COVID-19 by PCR (PCR+; n = 28) showed pronounced neutralizing activity against the SARS-CoV-2 S(G614) variant.

Neutralizing activity of monoclonal antibodies against SARS-CoV-2 variants

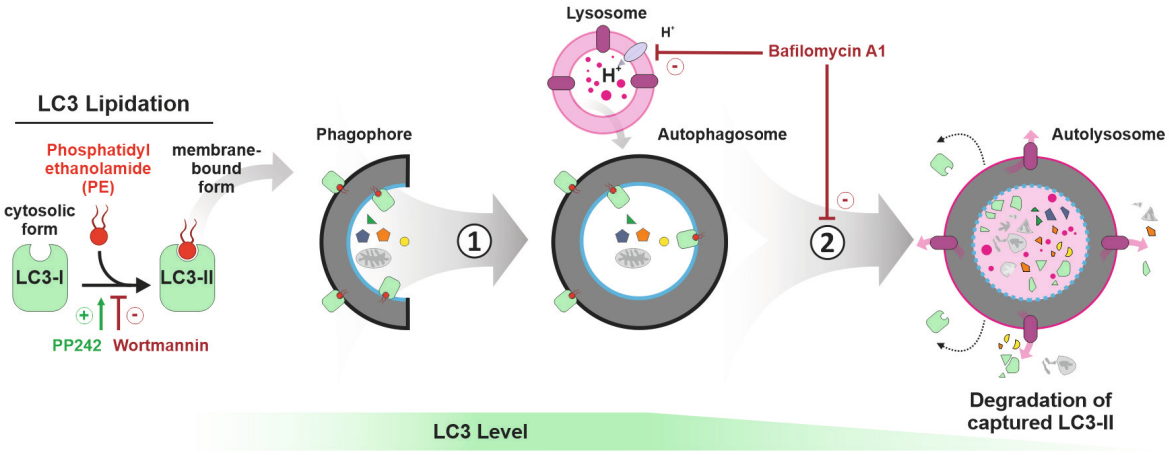


HiBiT-PsVLPs bearing either the wildtype (G614) or omicron SARS-CoV-2 spike protein were challenged (30 min) with a concentration series of four therapeutic anti-SARS-CoV-2 spike antibodies (Bamlanivimab, Etesevimba, Casirivimab, and Imdevimab). Following incubation (3 hours) with SARS-CoV-2 HEK293T Target Cells the luminescent signal determined using the *Nano-Glo® Live Cell Assay System*. None of the therapeutic antibodies showed neutralizing activity against SARS-CoV-2 omicron HiBiT PsVLPs while the positive control nAb (G10xA5) neutralized all variants.

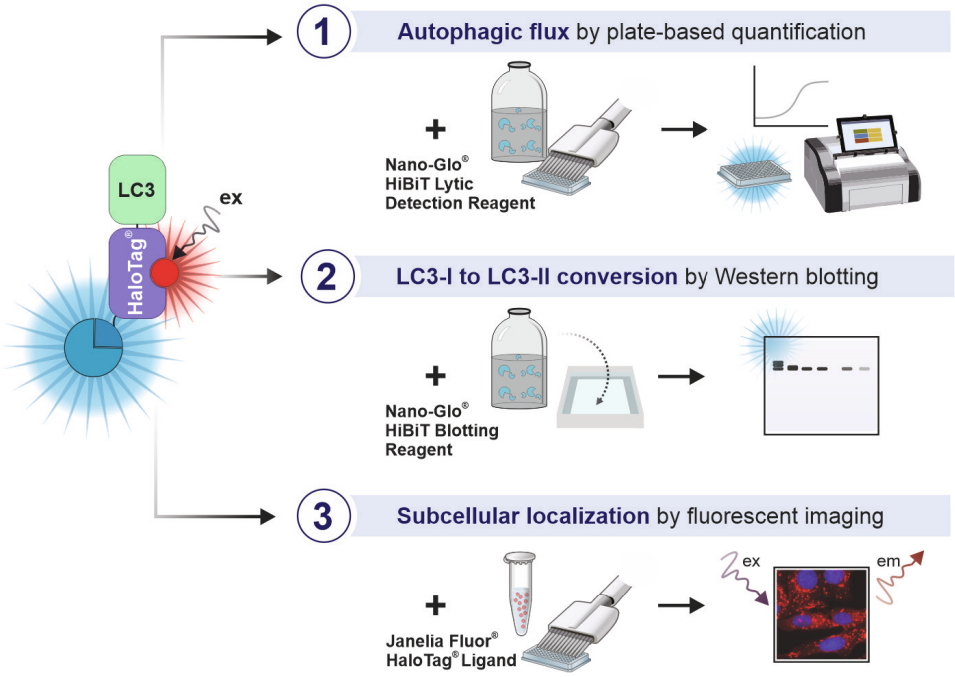
Illuminate Autophagy Dynamics

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

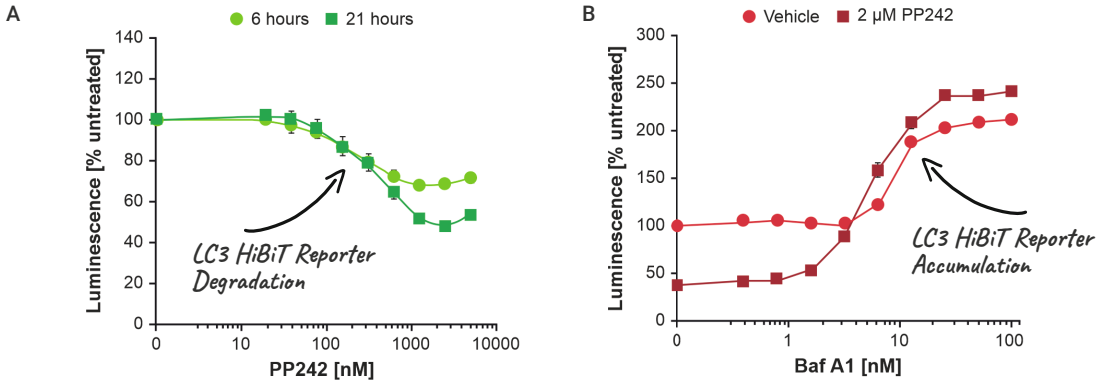


LC3 HiBiT Reporter – Three assays in one



The Autophagy LC3 HiBiT Reporter has been developed through the fusion of human LC3B to HiBiT and HaloTag®. **(1)** The level of LC3 HiBiT reporter is measured using a lytic luminescent plate-based assay. Luminescence intensity directly reflects the concentration of the reporter in cell lysate, meaning that the assay signal is inversely correlated with autophagic flux. **(2)** The conversion of LC3-I to LC3-II can be assessed in a convenient, antibody-free workflow using the Nano-Glo® HiBiT Blotting System. **(3)** Furthermore, the use of high-quantum-yield Janelia Fluor® HaloTag® Ligands facilitates fluorescent imaging of changes in LC3 reporter localization.

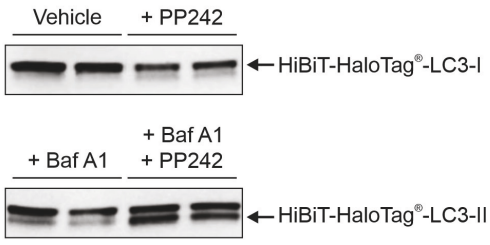
Measure autophagic flux



(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.

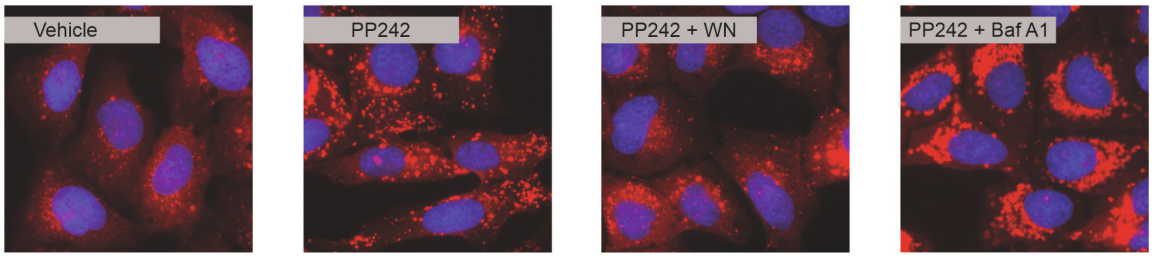
Measure LC3-I to LC3-II conversion

No antibodies required



U2OS Autophagy LC3 HiBiT Reporter Cells were treated with PP242 (autophagy stimulator) alone or with BafA1 (late-phase pathway inhibitor) for 4 hours. LC3-I and LC3-II HiBiT Reporters were resolved on a Bis Tris PAGE gel (10%), transferred to a nitrocellulose membrane, and examined using the Nano-Glo® HiBiT Blotting System. PP242 alone stimulates autophagy and increases overall degradation of the reporter. With the addition of Baf A1, LC3-I is converted to LC3-II but the degradation of LC3-II is blocked and both LC3-I and LC3-II levels increase.

Visualize LC3 localization to the autophagosome



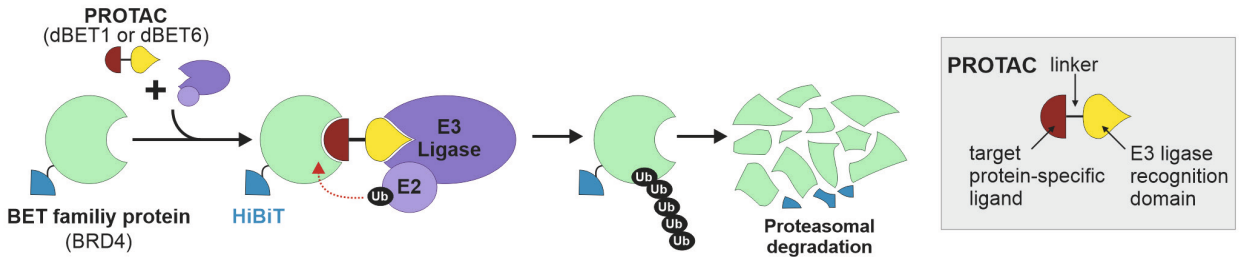
U20S Autophagy LC3 HiBiT Reporter Cells were treated for 4 hours with the autophagy stimulator PP242 and the autophagy inhibitors Wortmannin (WN) or Bafilomycin A1 (Baf A1). The localization of the LC3 reporter was imaged using Janelia Fluor® 646 HaloTag® Ligand. Images were acquired at 60X magnification. PP242 elevates autophagosome levels, which can be visualized as increased puncta compared to vehicle control. The early-phase pathway inhibitor WN prevents autophagosome formation (reduced puncta), while the late-phase pathway inhibitor Baf A1 elevates autophagosome formation (increased puncta).

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.

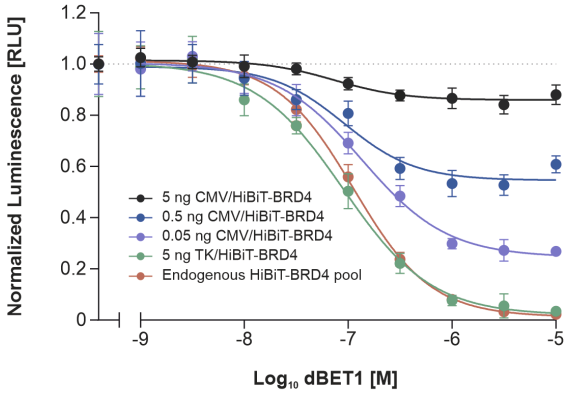
PROTACs – Targeting proteins for degradation

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.



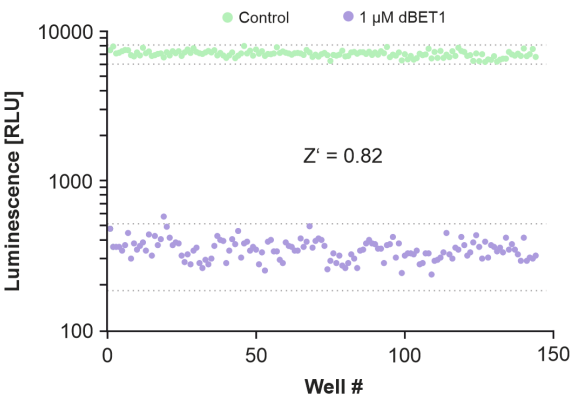
Monitoring targeted degradation of HiBiT-BRD4 in cells treated with the PROTAC dBET1

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.



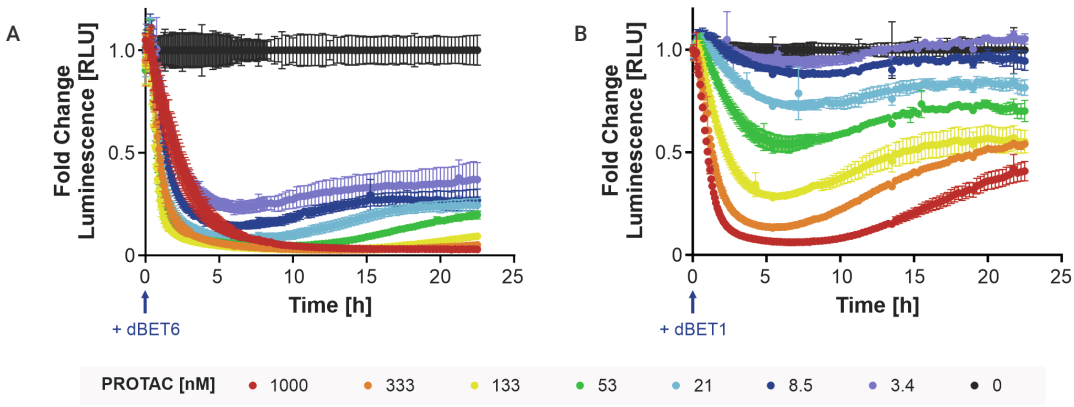
Induced degradation of HiBiT-BRD4 in a CRISPR-derived cell pool in 384-well plates

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.



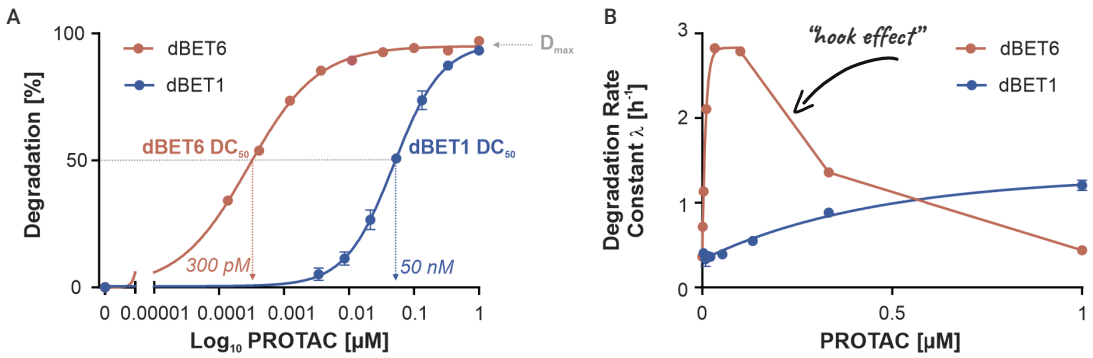
A New Way to Look at Protein Degradation

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

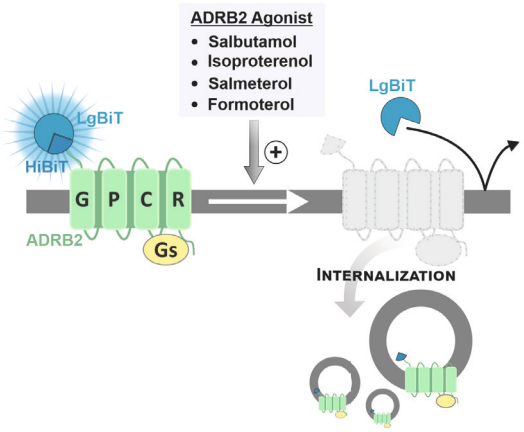
- PROTAC permeability
- Binary & ternary complex formation
- Ubiquitination
- Proteasomal recruitment

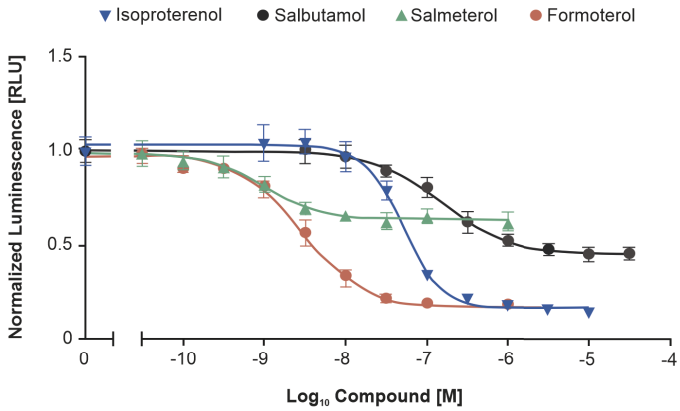
Surface Receptor Quantification & Internalization

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.



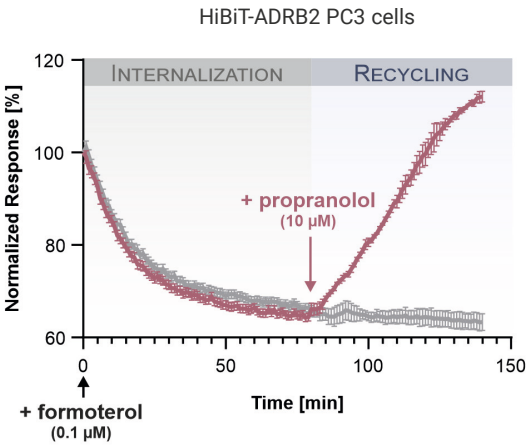


Agonist	EC_{50}	Percentage Receptor Remaining on Surface
Isoproterenol	50.9 nM	16 %
Salbutamol	161 nM	45 %
Salmeterol	1.04 nM	63 %
Formoterol	2.92 nM	16 %

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

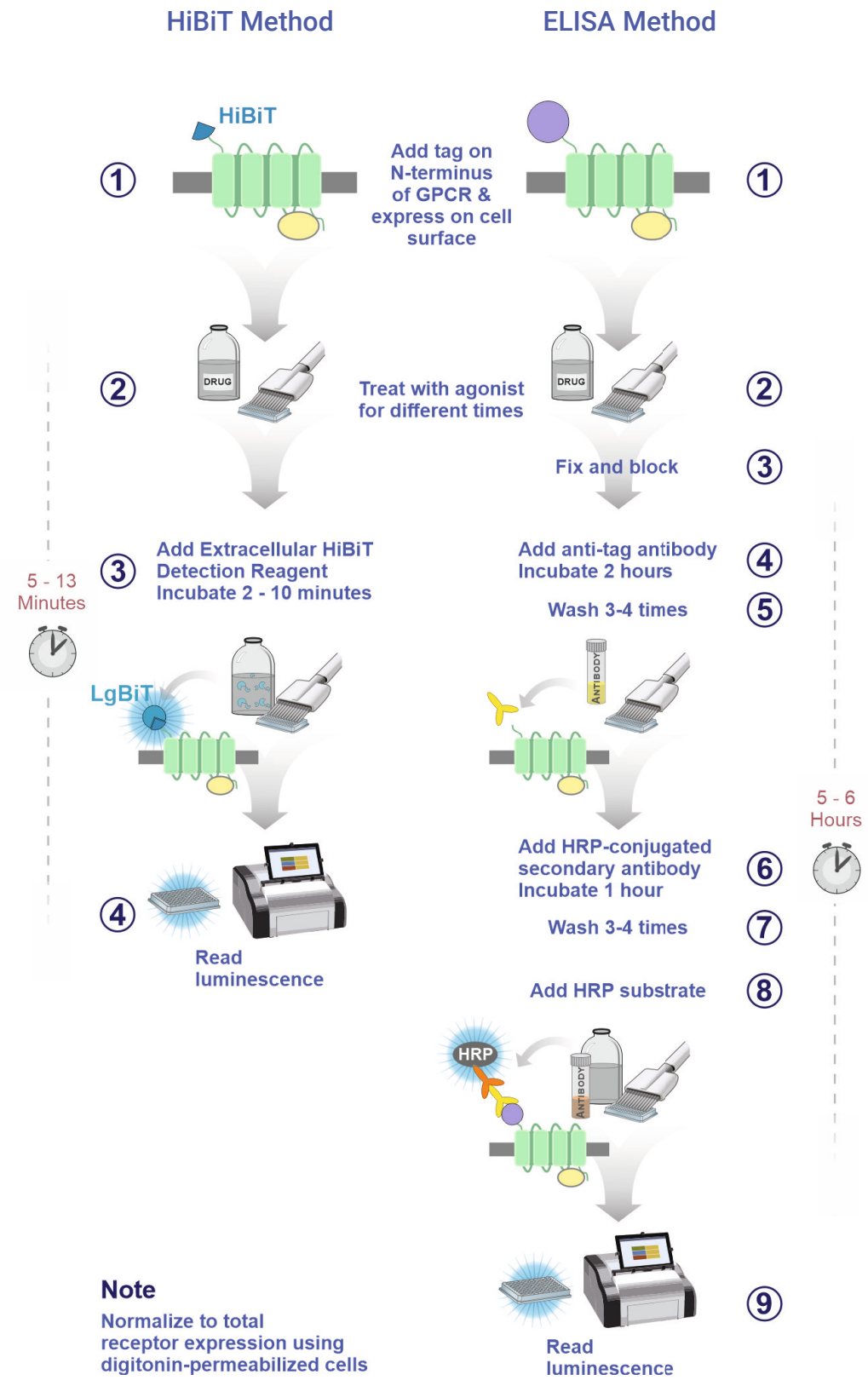
Addition of purified LgBiT Protein plus live-cell substrate (or extended substrate) enables real-time kinetic analysis of receptor trafficking even at endogenous expression levels. After pre-incubation of cells with LgBiT and substrate, the agonist formoterol induces ADRB2 receptor internalization. This 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.

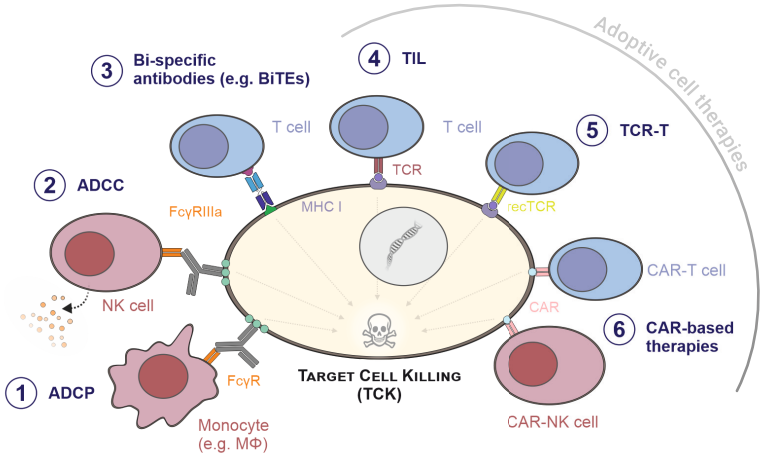


Determine Target Cell Killing in Co-Cultures

Target cell killing

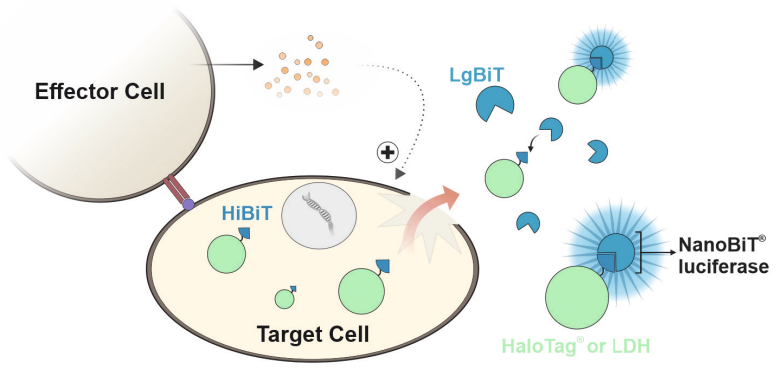
A promising new strategy to fight cancer is to harness the body's own immune system. Various approaches exist that are based on directing specific immune effector cells to kill the cancer target cells. Research in this area therefore requires technologies to specifically detect death of a target cell 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. Antibody-dependent cellular phagocytosis (ADCP)
2. Antibody-dependent cellular cytotoxicity (ADCC)
3. Bi-specific antibodies, e.g. bispecific T cell engager (BiTE)
4. Tumor-infiltrating lymphocytes (TIL)
5. T-cell receptor-engineered T cell therapy (TCR-T)
6. Chimeric antigen receptor-based therapies, e.g. CAR-T and CAR-NK cells



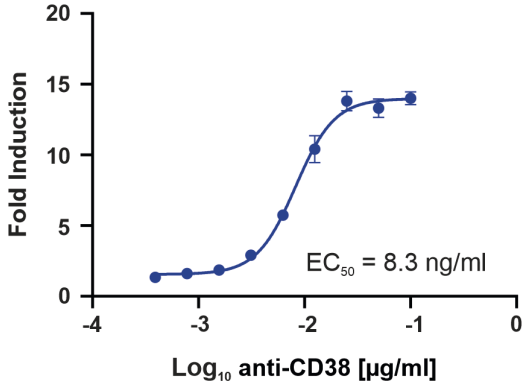
Principle of measuring target cell killing in co-cultures using HiBiT

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).

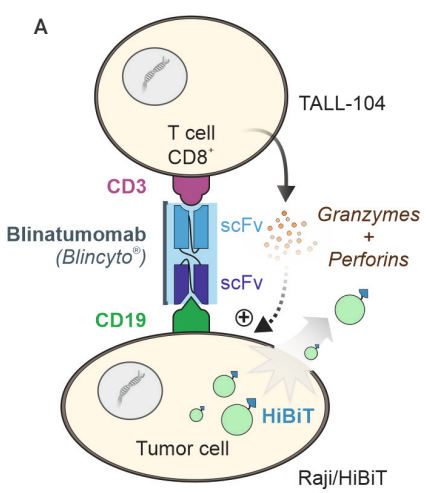


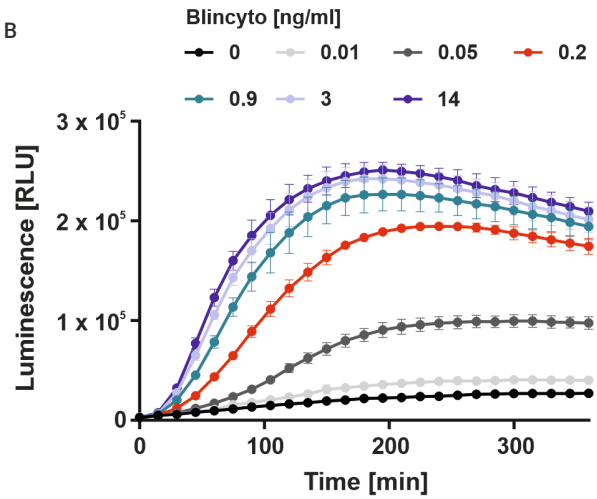
Antibody-dependent cellular cytotoxicity (ADCC)

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 *Bio-Glo-NB™ TCK Luciferase Assay System*.



T cell-dependent cellular cytotoxicity (TDCC)

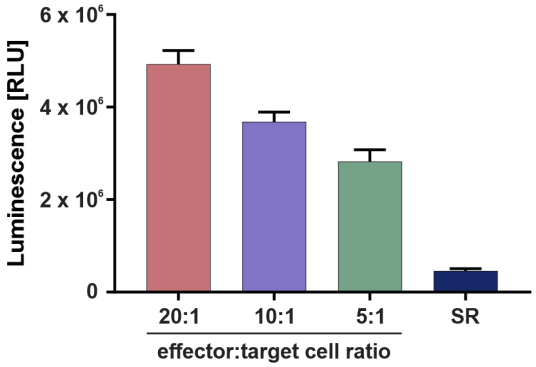




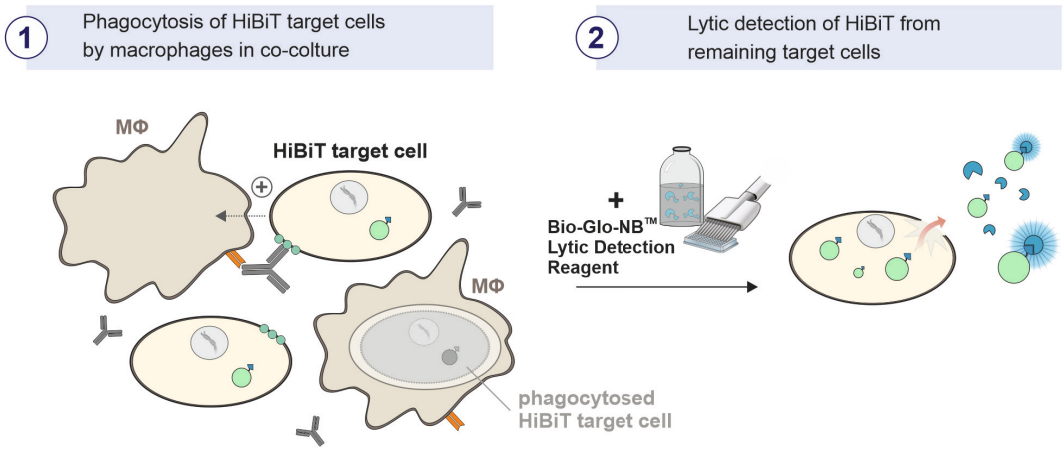
(A) Bi-specific T cell engagers (BiTEs) resemble a fusion of two single-chain variable fragments (scFv) of two different monoclonal antibodies. They redirect T cell cytotoxicity to tumor cells by simultaneous binding of a T cell- and a tumor-specific antigen. Blincyto®, for example, binds to CD3 on T 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 *Bio-Glo-NB™ TCK Luciferase Assay 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.

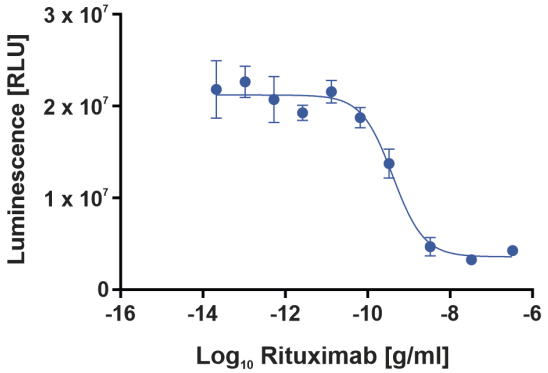


Principle of measuring target cell killing via ADCP using HiBiT



A co-culture of HiBiT target cells and primary macrophages is treated with a monoclonal antibody that triggers antibody-dependent cellular phagocytosis (ADCP). Upon phagocytosis, HiBiT target cells are subjected to lysosomal degradation. The luminescent signal from remaining target cells is detected in a lytic endpoint assay using the *Bio-Glo-NB™ Lytic Luciferase Assay System*. Therefore, ADCP activity correlates with reduced luminescence.

Antibody-dependent cellular phagocytosis (ADCP)



Stimulation of ADCP by Rituximab was analyzed using Ramos HaloTag®-HiBiT target cells. HiBiT target cells were added to primary human macrophage effector cells together with serial dilutions of Rituximab. Following incubation for 24 hours, luminescence was determined by addition of the *Bio-Glo-NB™Lytic Luciferase Assay System*.

The choice is yours – ready-to-use kits or build-your-own options

HiBiT Target Cell Killing Bioassay Kits

Standalone Components

HiBiT Target Cells	+	Primary Effector Cells	=	Target Cell Killing (TCK) Assay
Build-Your-Own or choose from different ready-to-use formats:		*Benefit from qualified primary effector cells for asured killing activity*		*Study TCK by different modes of action (MoA)*
• Cell Propagation Model (CPM) • Thaw-and-Use (TnU) • LDHB-HiBiT • HaloTag®-HiBiT • Negative control cells		• ADCC-qualified PBMC • TDCC-qualified T cells (CD8+) • ADCP-qualified Macrophages • CAR-T or TCR-T cells (*user-supplied*)		• ADCC • TDCC • ADCP • CAR-T or TCR-T cell-mediated

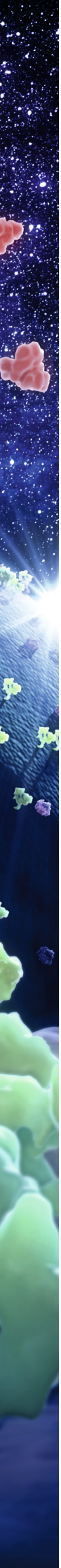
GloMax SYSTEMS

GloMax® Systems include **high-performance plate readers** with a range of detection options. From advanced multimode luminescence, fluorescence, absorbance, BRET and FRET capabilities.

	Luminescence	Fluorescence	Absorption visible	Absorption UV	BRET/FRET	Heating	Shaking	Plate formats
GloMax® Navigator	✔	✖	✖	✖	✖	✖	✖	96-well
GloMax® Explorer	✔	✔	optional	optional	optional	✔	✔	6- to 384-well
GloMax® Discover	✔	✔	✔	✔	✔	✔	✔	6- to 384-well

GloMax® Navigator GloMax® Explorer GloMax® Discover

For more information visit: www.promega.com/glomax-comparison



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

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

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)

Bio-Glo-NB™ TCK Luciferase Assay System

- Detection of released HiBiT in the HiBiT Target Cell Killing (TCK) assay

Cat. #	Quantity
CS3000A118 *	10 ml
CS3000A119 *	100 ml

Bio-Glo-NB™ Lytic Luciferase Assay System

- Detection of HiBiT in cell lysates from non-phagocytosed cells in the Macrophage ADCP Bioassay

Cat. #	Quantity
CS3055C06 *	10 ml
CS3055C07 *	100 ml

Anti-HiBiT Monoclonal Antibody

- Use as an orthogonal method to verify bioluminescent results
- Confirm subcellular localization by immunofluorescent imaging
- Confirm protein levels and size using classic Western blot detection
- Perform immunoprecipitation of HiBiT-tagged proteins
- Perform FACS analysis on live cells (extracellular HiBiT) or on fixed cells (intracellular HiBiT)

Cat. #	Quantity
N7200	100 µg
N7210	5 x 100 µg

Co-Expression of LgBiT Subunit

Product	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

* This is an Early Access Material. Please enquire for more information.

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® Vivazine™ Substrate

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

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

Nano-Glo® Endurazine™ Substrate

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

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
- 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

Cat. #	Quantity
N2590	0.1 ml each

LgBiT Protein

- Combine with Nano-Glo® Extended Live Cell Substrates for time course analysis of extracellular HiBiT fusion proteins

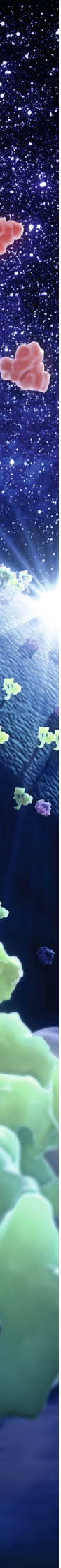
Cat. #	Quantity
CS1956A10 *	0.1 ml
CS1956A11 *	1 ml

* This is an Early Access Material. Please enquire for more information.

NanoBiT® Luciferase Inhibitors

Product	Cat. #	Quantity
Cell Permeable NanoBiT® Inhibitor	CS1576A02 *	100 µl (10 mM)
DrkBiT Peptide	CS3002A02 *	100 µl (1000X)

* This is an Early Access Material. Please enquire for more information.



HiBiT NanoDLR™

- Minimizing or eliminating experimental variability
- Control for specificity

Product	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

* This is an Early Access Material. Please enquire for more information.

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

Product	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

Product	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 **
HiBiT-HaloTag®-LC3B-LentiB3 Vector	CS1956B31 *	20 µg
Janelia Fluor® 503 HaloTag® Ligand	HT1010	5 pack, 1 nmol/vial
Janelia Fluor® 549 HaloTag® Ligand	HT1020	5 pack, 1 nmol/vial
Janelia Fluor® JFX554 HaloTag® Ligand	HT1030	5 pack, 1 nmol/vial
Janelia Fluor® 585 HaloTag® Ligand	HT1040	5 pack, 1 nmol/vial
Janelia Fluor® 635 HaloTag® Ligand	HT1050	5 pack, 1 nmol/vial
Janelia Fluor® 646 HaloTag® Ligand	HT1060	5 pack, 1 nmol/vial
Janelia Fluor® JFX650 HaloTag® Ligand	HT1070	5 pack, 1 nmol/vial
Janelia Fluor® HaloTag® Red Sample Pack	HT1100	3 pack, 1nmol/vial
Janelia Fluor® HaloTag® Far Red Sample Pack	HT1110	3 pack, 1nmol/vial

* This is an Early Access Material. Please enquire for more information.

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

HiBiT PsVLP Bioassays

SARS-CoV-2

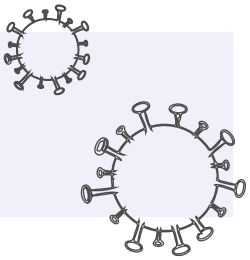
Product	Cat. #	Quantity
SARS-CoV-2 HiBiT-PsVLP Assay, Complete Kit *SARS-CoV-2 S(G614) HiBiT-PsVLPs included*	CS329524 *	1 kit
SARS-CoV-2 HiBiT-PsVLP Assay, Target Kit *No SARS-CoV-2 S HiBiT-PsVLPs included, complemented with standalone HiBiT-PsVLP variants*	CS329531 *	1 kit
SARS-CoV-2 S(G614) HiBiT-PsVLPs	CS329501 *	2 vials (120 assays)
SARS-CoV-2 S(Delta) HiBiT-PsVLPs	CS329509 *	2 vials (120 assays)
SARS-CoV-2 S(Omicron) HiBiT-PsVLPs	CS329533 *	2 vials (120 assays)
SARS-CoV-2 S(Omicron BA.4_5) HiBiT-PsVLPs	CS329537 *	2 vials (120 assays)
SARS-CoV-2 S(XBB.1.5) HiBiT-PsVLPs	CS329540 *	2 vials (120 assays)
VSV-G HiBiT PsVLPs *Specificity control*	CS329517 *	2 vials (120 assays)
SARS-CoV-2 HEK293T(LgBiT) Target Cells, Thaw-and-use	CS329520 *	1 vial (120 assays)
SARS-CoV-2 HEK293T(LgBiT) Target Cells, CPM	CS329523 *	2 vials

* This is an Early Access Material. Please enquire for more information

HiBiT-PsVLPs with spike protein from other SARS-CoV-2 variants available

e.g. alpha (α), beta (β), gamma (γ), epsilon (ε), iota (ι), kappa (κ) and lambda (λ)

Please enquire.



Ebola Virus (EBOV)

Product	Cat. #	Quantity
EBOV GP HiBiT-PsVLP, Complete Kit *EBOV GP HiBiT-PsVLPs included*	CS3368A09 *	1 kit
EBOV HiBiT-PsVLP Assay, Target Kit *No EBOV GP HiBiT-PsVLPs included, complemented with standalone HiBiT-PsVLP variants*	CS3368A11 *	1 kit
EBOV GP HiBiT-PsVLPs, 1X	CS3368A04 *	2 vials (120 assays)
EBOV HEK293T (LgBiT) Target Cells, Thaw-and-use	CS3368A07 *	1 vial (120 assays)
EBOV HEK293T (LgBiT) Target Cells, CPM	CS3368A02 *	2 vials

* This is an Early Access Material. Please enquire for more information.

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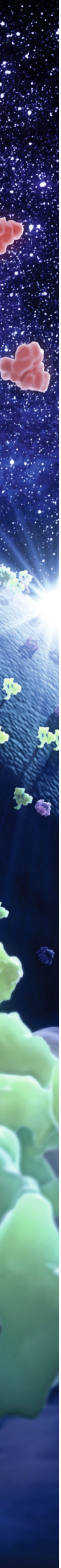

HiBiT Target Cell Killing Assay

HaloTag®-HiBiT Expression Vectors

- Create HiBiT target cells with your cell backtground of choice

Product	Cat. #	Quantity
HaloTag®-HiBiT Vector [CAG / Blast]	CS1956B17 *	20 µg
HaloTag®-HiBiT-LentiB4 Transfer Vector	CS3055A56 *	20 µg

* This is an Early Access Material. Please enquire for more information.

HaloTag®-HiBiT Target Cells

Product	Therapeutic target(s)	Cat. #	Quantity
A375 Cells, CPM	*MHCII*	CS3000A83 *	2 vials
A375 Cells, Thaw-and-use	*MHCII*	CS3000A80 *	1 vial
A549 Cells, CPM	*EGFR*	CS3055A10 *	2 vials
A549 Cells, Thaw-and-use	*EGFR*	CS3055A08 *	1 vial
CHO-K1 Cells, CPM	*Used as negative control*	please enquire	2 vials
CHO-K1 Cells, Thaw-and-use	*Used as negative control*	CS3055A60 *	1 vial
CHO-K1 Claudin 18.2 Cells, CPM	*Claudin 18.2*	CS347203 *	2 vials
CHO-K1 Claudin 18.2 Cells, Thaw-and-use	*Claudin 18.2*	CS347204 *	1 vial
CHO-K1 Membrane TNFα, CPM	*Membrane TNFα*	CS3055A44 *	2 vials
CHO-K1 Membrane TNFα, Thaw-and-use	*Membrane TNFα*	CS3055A46 *	1 vial
CHO-K1 SARS-CoV-2 S Cells, CPM	*SARS-CoV-2 Spike (S) protein*	CS3195A04 *	2 vials
CHO-K1 SARS-CoV-2 S Cells, Thaw-and-use	*SARS-CoV-2 Spike (S) protein*	CS3195A01 *	1 vial
CHO-K1 TIGIT Cells, CPM	*TIGIT*	CS3000A78 *	2 vials
CHO-K1 TIGIT Cells, Thaw-and-use	*TIGIT*	CS3000A75 *	1 vial
H929 Cells, CPM	*BCMA, CD38, CD52, CD138*	CS3055A13 *	2 vials
H929 Cells, Thaw-and-use	*BCMA, CD38, CD52, CD138*	CS3055A11	1 vial
K562 BCMA Cells, CPM	*BCMA*	CS3000A16 *	2 vials
K562 BCMA Cells, Thaw-and-use	*BCMA*	please enquire	1 vial
K562 CD19 Cells, CPM	*CD19*	CS3000A25 *	2 vials
K562 CD19 Cells, Thaw-and-use	*CD19*	please enquire	1 vial
K562 Cells, CPM	*Used as negative control*	CS3000A50 *	2 vials
K562 Cells, Thaw-and-use	*Used as negative control*	CS3000A52 *	1 vial
K562 CIITA Cells, CPM	*CIITA*	CS3000A59 *	2 vials
K562 CIITA Cells, Thaw-and-use	*CIITA*	CS3000A61 *	1 vial
K562 GPC3 Cells, CPM	*GPC3*	CS3000A64 *	2 vials
K562 GPC3 Cells, Thaw-and-use	*GPC3*	CS3000A66 *	1 vial
Raji Cells, CPM	*CD19, CD20, CD22, CD38, CD70*	CS3055A04 *	2 vials
Raji Cells, Thaw-and-use	*CD19, CD20, CD22, CD38, CD70*	CS3055A02 *	1 vial
Ramos Cells, CPM	*CD19, CD20, CD22, CD38, CD52*	CS3055A07 *	2 vials
Ramos Cells, Thaw-and-use	*CD19, CD20, CD22, CD38, CD52*	CS3055A05 *	1 vial
SK-BR-3 Cells, CPM	*HER2, EpCAM*	CS3055A33 *	2 vials
SK-BR-3 Cells, Thaw-and-use	*HER2, EpCAM*	CS3055A35 *	1 vial
U937, CPM	*CD33, CLL-1, CD123*	CS3000A08 *	2 vials
U937, Thaw-and-use	*CD33, CLL-1, CD123*	please enquire	1 vial

* This is an Early Access Material. Please enquire for more information.

LDH-HiBiT Target Cells

Product	Therapeutic target(s)	Cat. #	Quantity
OVCAR3 Cells, CPM	*MSLN, 5T4, WT, HER2*	CS3000A29 *	2 vials
OVCAR3 Cells, Thaw-and-use	*MSLN, 5T4, WT, HER2*	CS3000A40 *	1 vial
Raji CD19-KO CD20-KO Cells, CPM	*CD22, CD38, CD70*	CS3000A57 *	2 vials
Raji CD19-KO CD20-KO Cells, Thaw-and-use	*CD22, CD38, CD70*	CS3000A72 *	1 vial
Raji CD19-KO Cells, CPM	*CD20, CD22, CD38, CD70*	CS3000A20 *	2 vials
Raji CD19-KO Cells, Thaw-and-use	*CD20, CD22, CD38, CD70*	CS3000A47 *	1 vial
Raji CD20-KO Cells, CPM	*CD19, CD22, CD38, CD70*	CS3000A56 *	2 vials
Raji CD20-KO Cells, Thaw-and-use	*CD19, CD22, CD38, CD70*	CS3000A69 *	1 vial
Raji Cells, CPM	*CD19, CD20, CD22, CD38, CD70*	CS3000A18 *	2 vials
Raji Cells, Thaw-and-use	*CD19, CD20, CD22, CD38, CD70*	CS3000A45 *	1 vial
Ramos CD19-KO Cells, CPM	*CD20, CD22, CD38, CD52*	CS3000A24 *	2 vials
Ramos CD19-KO Cells, Thaw-and-use	*CD20, CD22, CD38, CD52*	please enquire	1 vial
Ramos Cells, CPM	*CD19, CD20, CD22, CD38, CD52*	CS3000A22 *	2 vials
Ramos Cells, Thaw-and-use	*CD19, CD20, CD22, CD38, CD52*	please enquire	1 vial
SKOV3 Cells, CPM	*MSLN, 5T4, MUC16, HER2*	CS3000A33 *	2 vials
SKOV3 Cells, Thaw-and-use	*MSLN, 5T4, MUC16, HER2*	CS3000A43 *	1 vial
T2 Cells, CPM	*HLA-A2+, CD5, CD7, CD30, CD52*	CS3000A35 *	2 vials
T2 Cells, Thaw-and-use	*HLA-A2+, CD5, CD7, CD30, CD52*	please enquire	1 vial

* This is an Early Access Material. Please enquire for more information.

Human Primary Effector Cell Bioassays

Product	Cat. #	Quantity
PBMC ADCC Bioassay Kit (Raji)	CS3055A14 *	1 kit **
PBMC ADCC Bioassay Kit (Ramos)	CS3055A20 *	1 kit **
PBMC ADCC Bioassay Kit (SK-BR-3)	CS3055A36 *	1 kit **
PBMC ADCC Bioassay Kit (A549)	CS3055A24 *	1 kit **
PBMC ADCC Bioassay Kit (H929)	CS3055A28 *	1 kit **
PBMC ADCC Bioassay Kit (SKOV3)	CS3055A51 *	1 kit **
Human PBMC, ADCC Qualified	CS3055A01 *	1 vial
Human T Cell (CD8+) TDCC Bioassay Kit (Raji)	CS3055B16 *	1 kit **
Human T Cell (CD8+) TDCC Bioassay Kit (Ramos)	CS3055B02 *	1 kit **
Human T Cell (CD8+) TDCC Bioassay Kit (SK-BR-3)	CS3055B10 *	1 kit **
Human T Cell TDCC Bioassay Kit (A549)	CS3055B12 *	1 kit **
Human T Cell (CD8+) TDCC Bioassay Kit (H929)	CS3055B08 *	1 kit **
Human T Cell TDCC Bioassay Kit (SKOV3)	CS3055B14 *	1 kit **
Human T Cell (CD8+), TDCC Qualified	CS3055B03 *	1 vial
Human Macrophages, ADCP Qualified	CS3055C02 *	1 vial

* This is an Early Access Material. Please enquire for more information.

** Cell culture medium, FBS and 10 ml Bio-Glo-NB™ TCK Luciferase Assay System included

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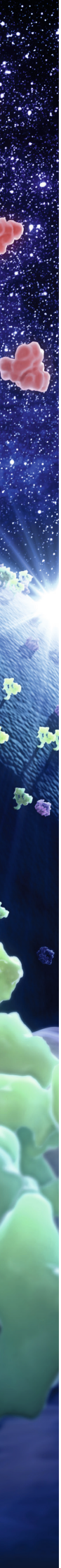

Notes

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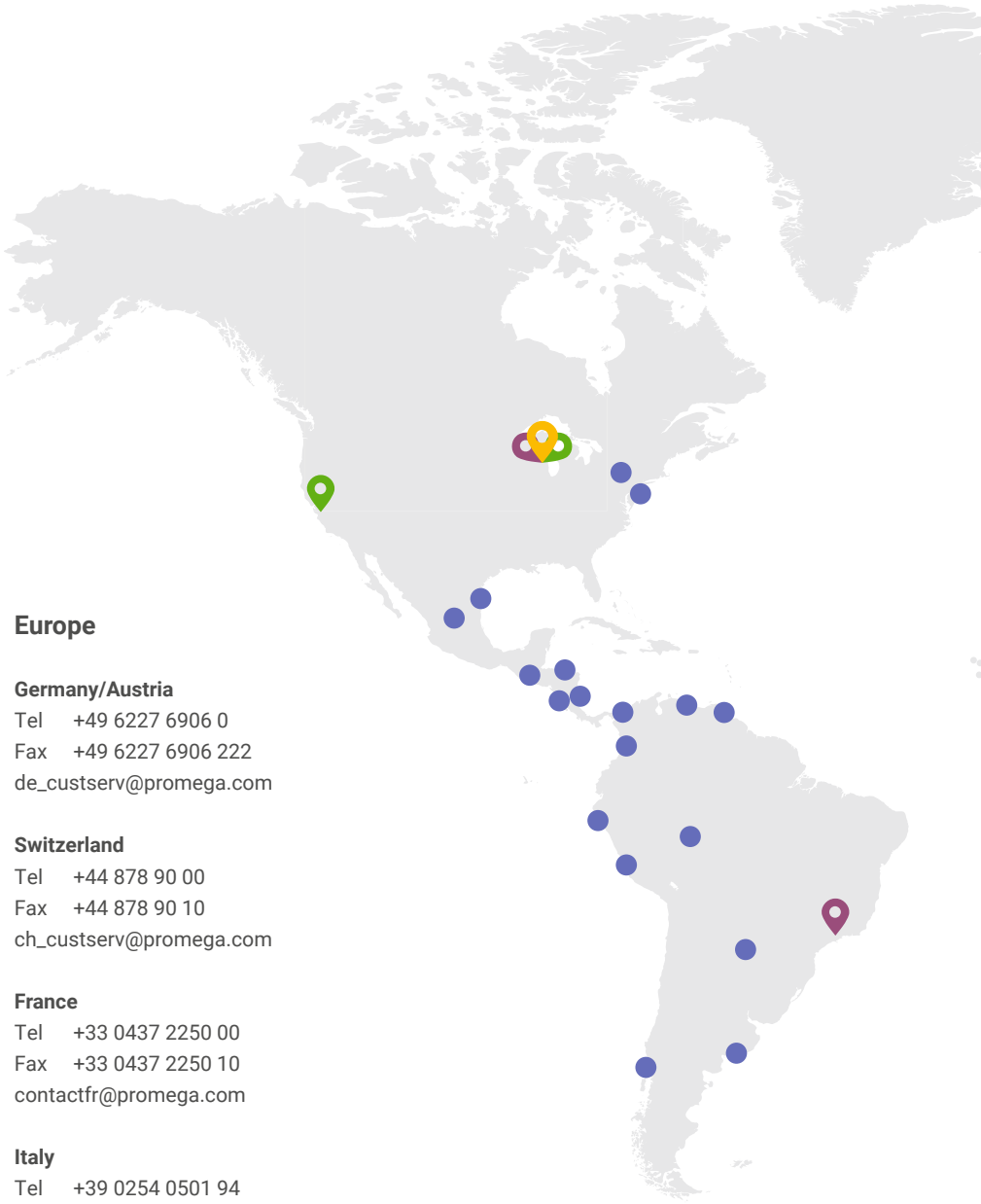

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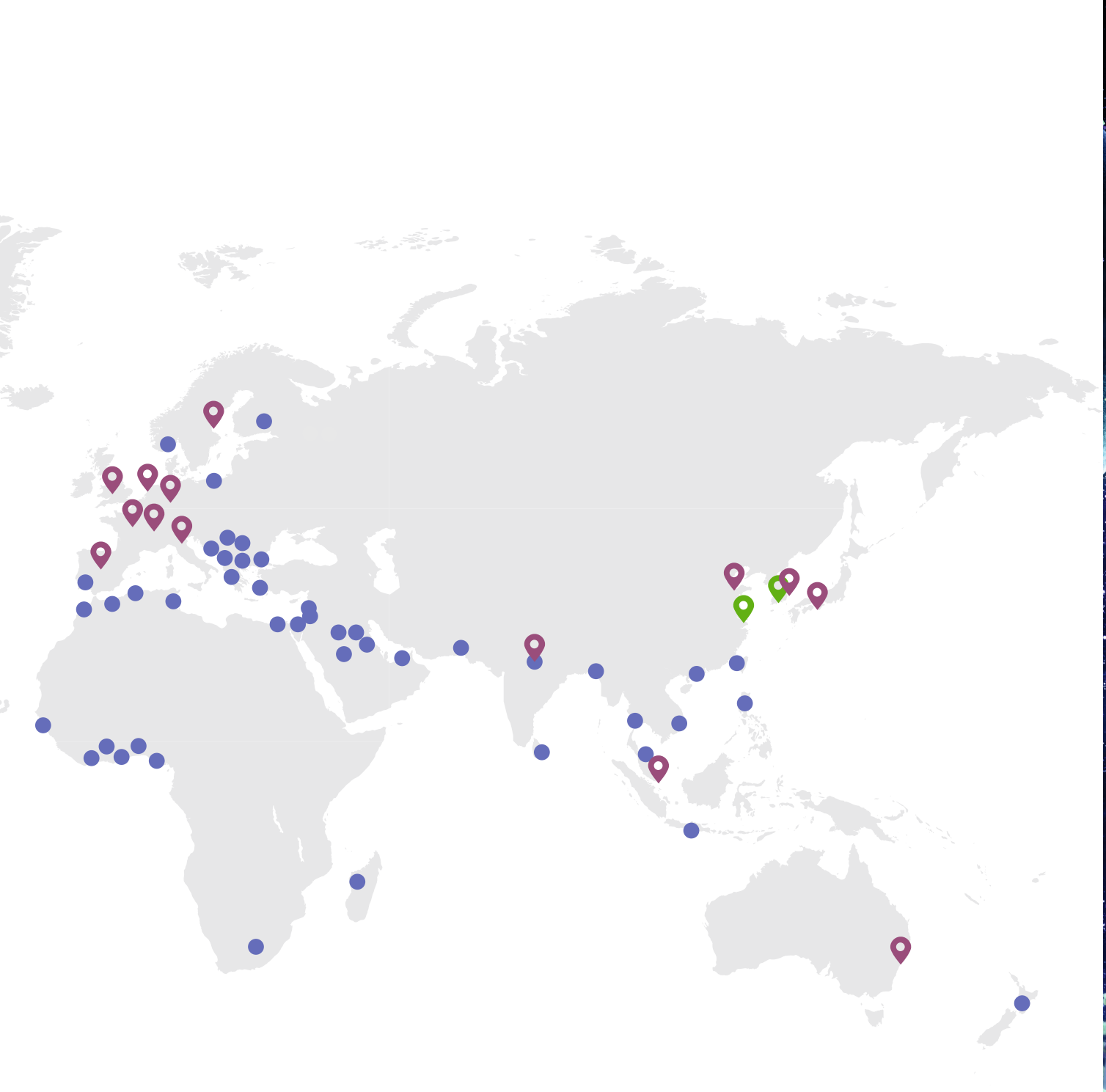

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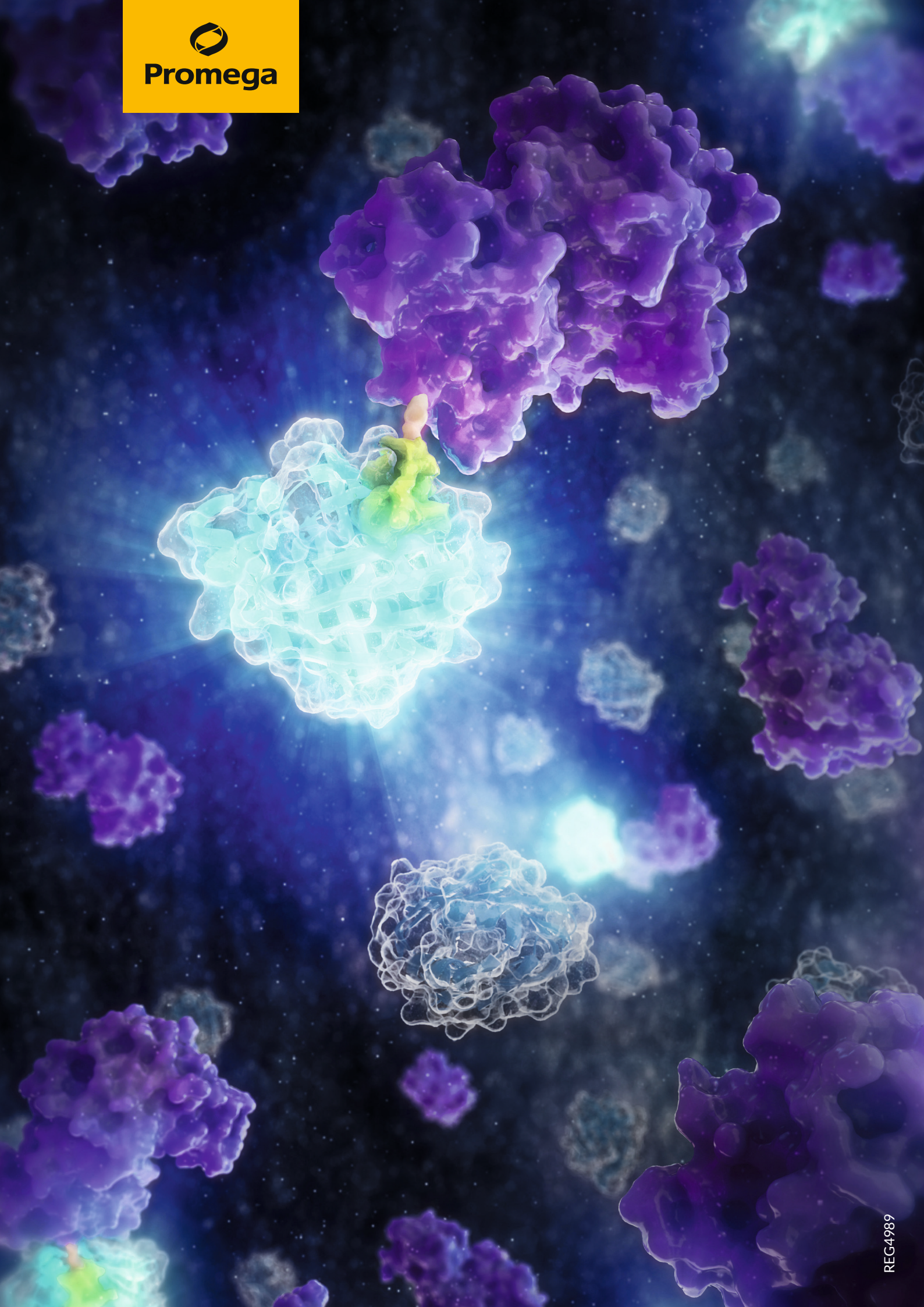
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