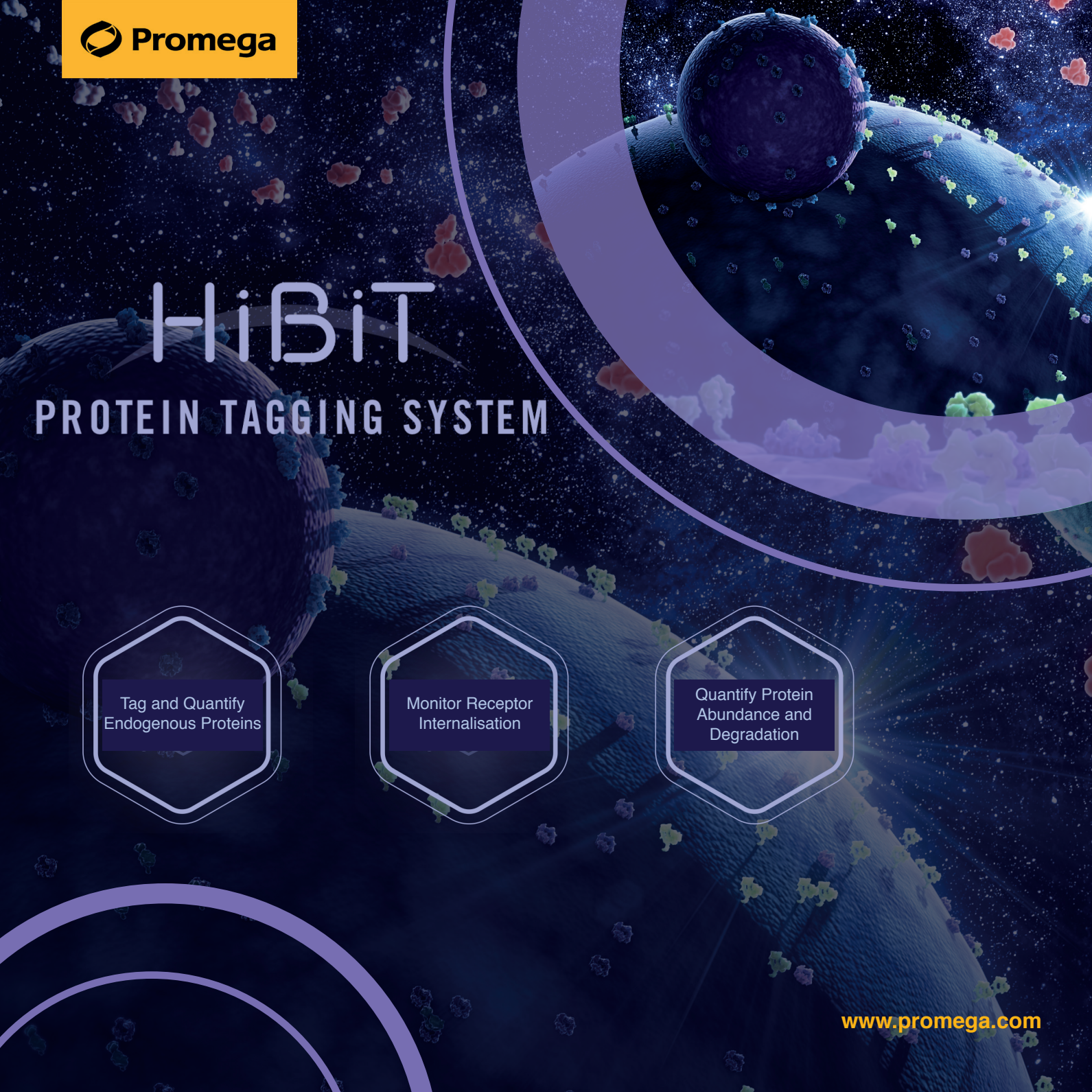
Promega

HiBiT

PROTEIN TAGGING SYSTEM



Tag and Quantify Endogenous Proteins

Monitor Receptor Internalisation

Quantify Protein Abundance and Degradation

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Simplify protein tagging

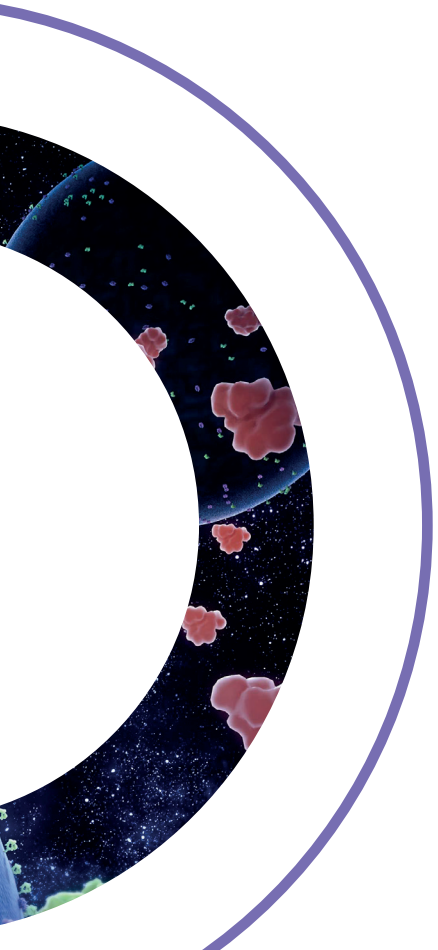
Detection and quantitation with a streamlined, antibody-free protocol

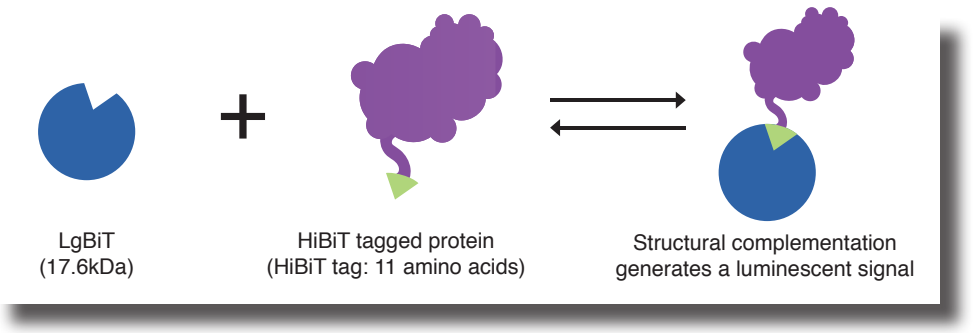
With the sensitivity to detect proteins without overexpression and the convenience of a single-reagent-addition method

Understanding changes in cellular protein abundance is key to understanding basic cellular biology and the dysregulation that can underlie many disease states. HiBiT protein tagging technology opens up a universe of possibilities for researchers to study protein biology.

HiBiT Bioluminescent Tagging System

HiBiT tagging technology is a binary complementation system based on NanoBiT® luciferase, which enables the sensitive, bioluminescent quantitation of proteins. Due to its small size (11 amino acids), the HiBiT peptide tag has very little effect on target protein function. HiBiT-tagged proteins are measured via detection reagents containing its complementary polypeptide Large BiT (LgBiT). HiBiT and LgBiT spontaneously bind with high affinity (K_D~1nM) to reconstitute an active enzyme, which in the presence of substrate, generates a bright luminescent signal.





Principle of HiBiT technology. HiBiT tagged to the protein of interest interacts spontaneously with its complementary polypeptide LgBiT forming an active luciferase enzyme, which in the presence of its substrate produces a very bright luminescent signal.

HiBiT tagging enables simple, bioluminescent quantitation of total or surface-expressed proteins, with a wide dynamic range and sensitivity compatible with detection of endogenously expressed proteins. HiBiT can also be used to detect protein expression in CRISPR gene editing systems.

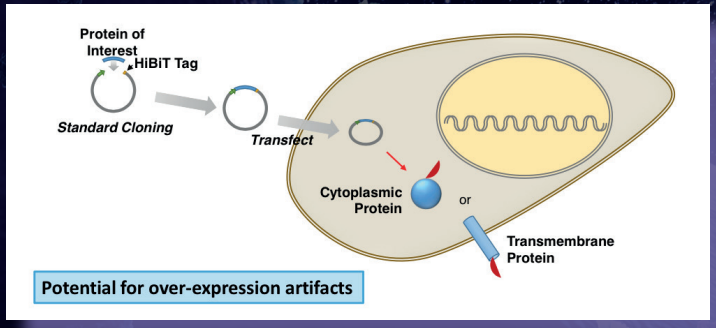
HiBiT technology offers:

- Very small (11 amino acid) tag
- Sensitive, bright luminescent output
- Linear dynamic range (7 logs)
- Compatible with standard cloning approaches and CRISPR/Cas9 genome editing
- Antibody-free, simple, single-reagent-addition protocols
- Scalability from bench to high-throughput screening

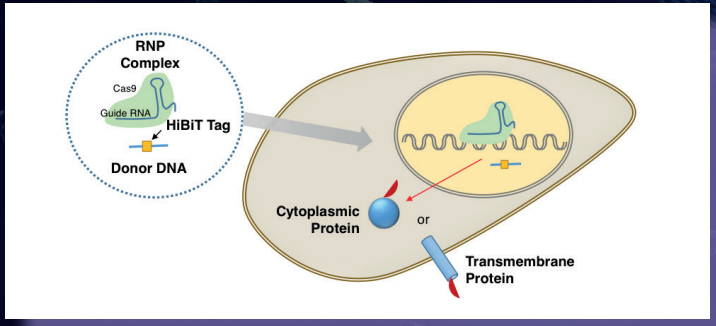
HiBiT tagging: use in cloning or CRISPR/Cas9 systems

HiBiT protein tag can be used in both standard cloning and CRISPR/Cas9 editing systems. The small size of HiBiT has little impact on target protein function and biology.

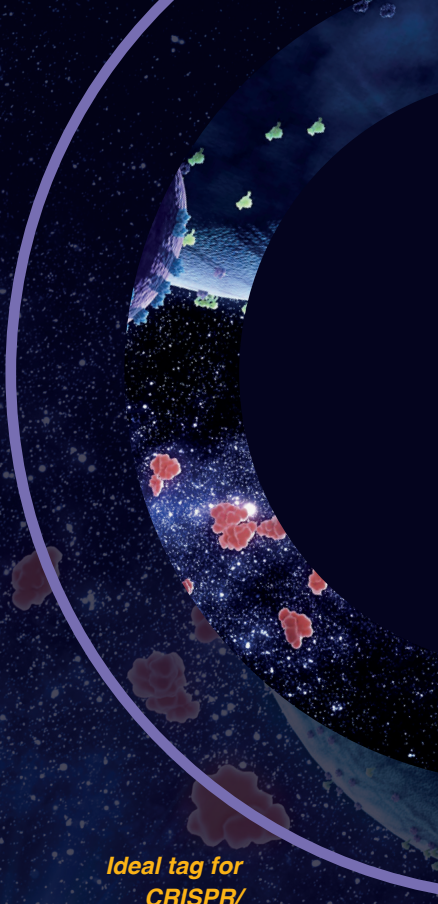
Standard cloning



CRISPR/Cas9 editing



Summary of two options for tagging HiBiT to proteins of interest



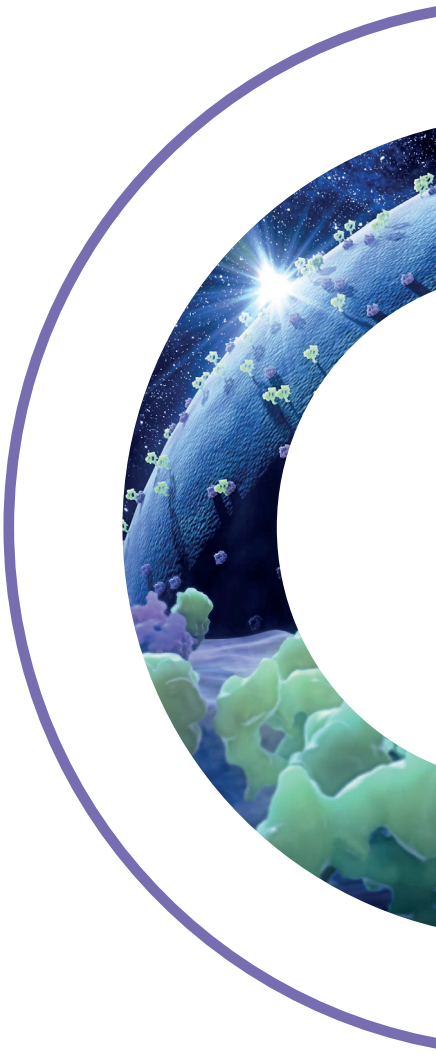
Ideal tag for CRISPR/ Cas9-mediated genome editing

Detection of HiBiT-tagged proteins

There are three different approaches to measure tagged proteins, each using a different Nano-Glo detection reagent, all containing the enzyme substrate and purified LgBiT.

Detection System	Description	Applications
Lytic Nano-Glo® HiBiT Lytic Detection Reagent	Sensitive bioluminescent protein detection measured directly in cell lysates	• Regulated protein expression • Targeted protein degradation • Viral infection • Verification of protein levels from gene expression and RNA-seq experiments • Protein abundance following pull-downs or transient transfection
Non-lytic Nano-Glo® HiBiT Extracellular Detection Reagent	Specific, live-cell, real-time detection of surface-expressed or secreted proteins	• Receptor internalisation • Receptor recycling • Protein or cytokine secretion • Surface protein trafficking
Blotting Nano-Glo® HiBiT Blotting System	Fast, antibody-free alternative to western blotting for detection of blotted proteins	• Verification of molecular weight of HiBiT-tagged proteins • Identification of splice variant expression • Quick screening of transient transfections or CRISPR knock-ins

Summary of HiBiT applications by product name



Recombinant expression of HiBiT-tagged proteins

A range of HiBiT fusion vectors is available to support the generation of HiBiT-tagged proteins. The gene of interest is easily introduced into one of the HiBiT expression vectors following standard cloning protocols. HiBiT vectors enable addition of the HiBIT tag to the N- or C-terminus of the protein of interest. The HiBiT tag can also be added directly to existing protein expression constructs by PCR-based or gene-synthesis methods.

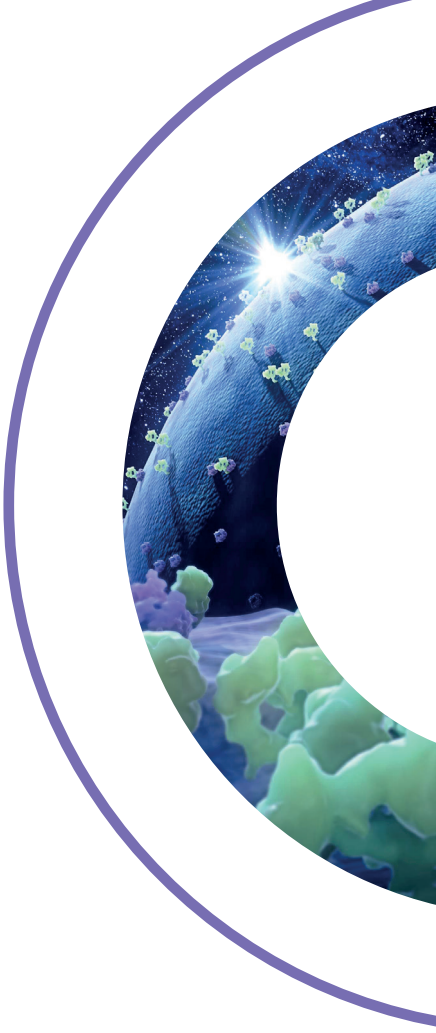
Ordering information

Description	Cloning Format	Tag orientation	Cat No.
pBiT3.1-N [CMV/HiBiT/Blast]	MSC	HiBiT-POI	N2361
pBiT3.1-C [CMV/HiBiT/Blast]	MSC	POI-HiBiT	N2371
pBiT3.1-secN [CMV/HiBiT/Blast]	MSC	IL6-HiBiT-POI	N2381
pFC37K HiBiT CMV-neo Flexi® Vector	Flexi	POI-HiBiT	N2391
pFN38K HiBiT CMV-neo Flexi® Vector	Flexi	HiBiT-POI	N2401
pFN39K secHiBiT CMV-neo Flexi® Vector	Flexi	IL6-HiBiT-POI	N2411

POI- Protein of interest

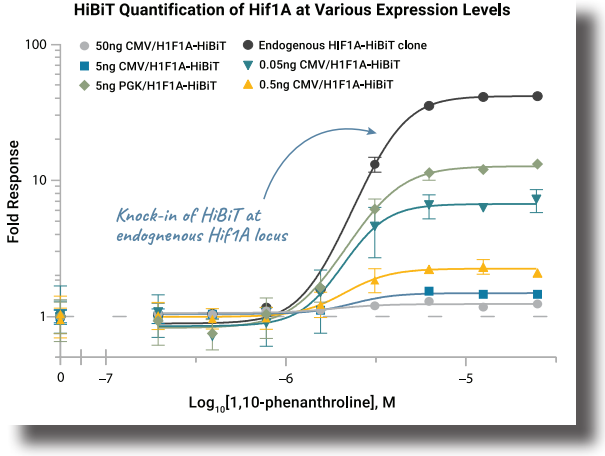
CRISPR/Cas9 knock-in of HiBiT at the endogenous locus

CRISPR/Cas9 technology can be used to insert or ‘knock in’ the HiBiT tag sequence at a precise location within a gene locus. This approach enables the study of endogenous proteins expressed under native regulatory conditions, reducing overexpression artifacts and maintaining proper stoichiometry with other endogenous binding partners or regulatory machinery. The small size of the HiBiT tag (11 amino acids) gives high insertion rates compared to bulky reporters such as GFP, and minimises any potential interference with normal target protein function.



Tagging endogenous proteins with HiBiT using CRISPR:

- Tag low-abundance endogenous proteins
- Small tag preserves native biology of the target protein
- Highly efficient workflow eliminates cloning
- Results can be measured 24-48 hours post-editing
- High sensitivity, eliminating the need for over-expression
- Quantify expression dynamics in real time
- Excellent results in primary cells (See Schwinn et al. 2017)

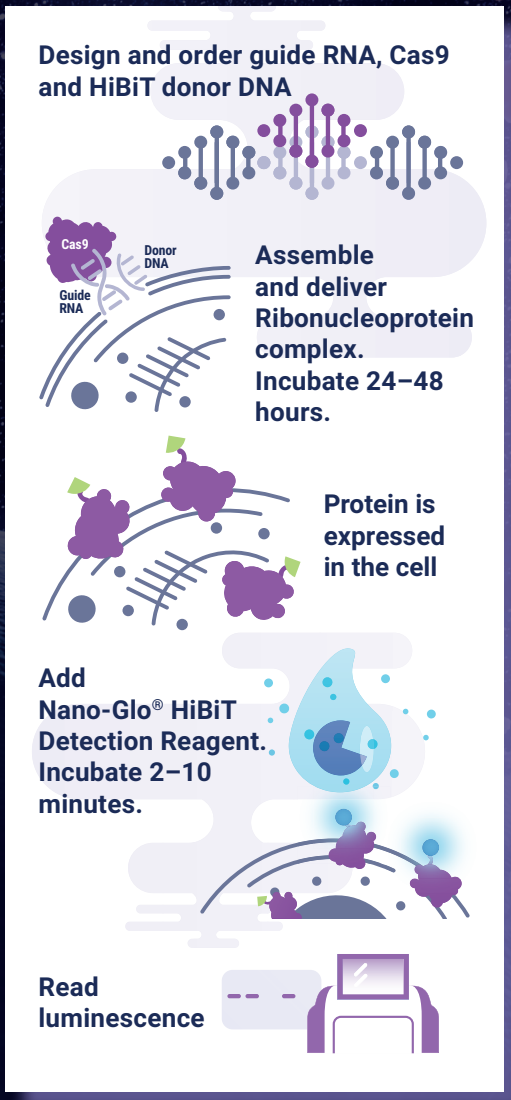


HeLa cells were transiently transfected with different amounts of CMV- or PGK-driven expression constructs for HIF1A-HiBiT, diluted in carrier DNA. In parallel, HiBiT was tagged to the endogenous locus in HeLa cells using CRISPR/Cas9, and a clone was isolated. Cells were treated for 4 hours with a titration of 1,10-phenanthroline. Nano-Glo® HiBiT Lytic Reagent was added to all wells, and luminescence was measured after 10 minutes.

For more information on this application, visit Promega.co.uk and download:

- Rapid Clone-Free CRISPR/Cas9 protocol for HiBiT Knock-In
- Schwinn, M.K. et al. (2017) CRISPR-mediated tagging of endogenous proteins with a luminescent peptide. ACS Chem. Biol.

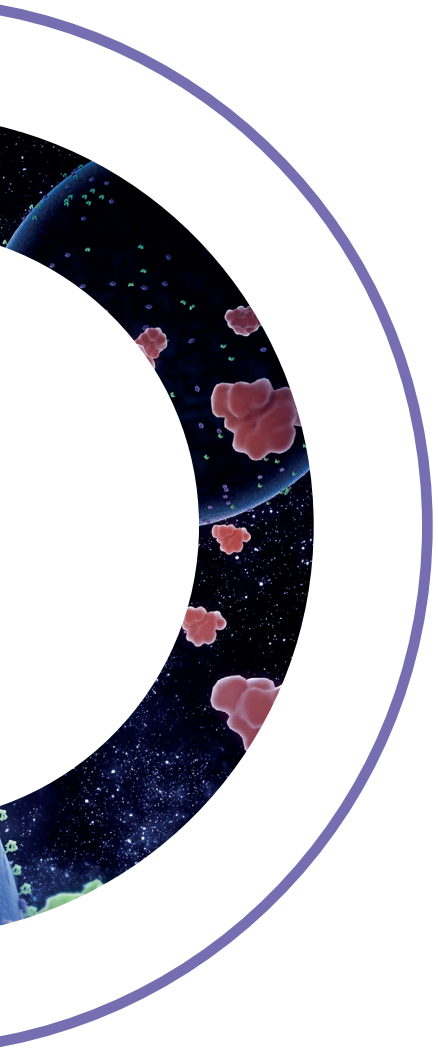
Protocol for adding the HiBiT tag to an endogenous gene using CRISPR gene editing



1. Identify target and find genomic sequence
2. Design crRNA and order guide RNA (crRNA + tracrRNA)
3. Design and order HiBiT Donor DNA template
4. Obtain synthesis rights and access HiBiT sequence from: www.promega.com/HiBiT-synthesis
5. Deliver guide RNA, donor DNA and Cas9
6. Validate editing event in cells

Nano-Glo HiBiT Lytic Detection System

Sensitive detection of HiBiT-tagged proteins in cell lysates



Nano-Glo HiBiT Lytic Detection System is a sensitive, bioluminescent method for the direct detection and quantitation of HiBiT-tagged proteins in cell lysates.

The HiBiT peptide tag is added to the protein of interest through traditional cloning methods or CRISPR/Cas9 genome editing techniques and once expressed, the total amount of HiBiT-tagged protein is measured by adding the Nano-Glo HiBiT Lytic Detection Reagent.

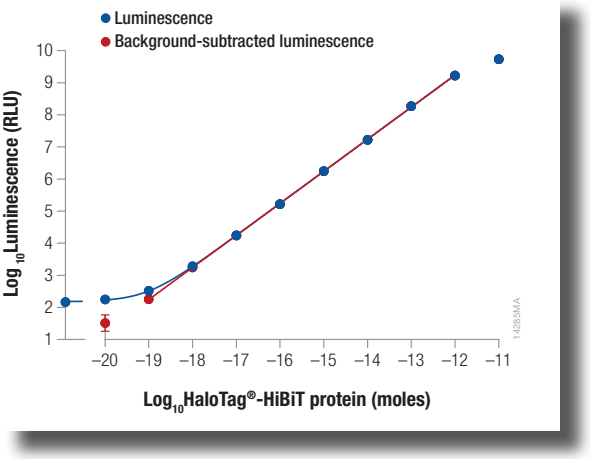
Nano-Glo HiBiT Lytic Detection Reagent consists of a detergent-containing buffer for lysis of cell membranes, a luciferase substrate and the cell-impermeable polypeptide LgBiT. Upon cell lysis, LgBiT spontaneously interacts with the HiBiT tag reconstituting the active luciferase enzyme. The amount of luminescence generated by the luciferase is directly proportional to the amount of HiBiT-tagged protein in the cell lysate over 7 orders of magnitude. Assay results can be obtained in just 10 minutes using a luminometer and the glow-type signal is stable for hours.

Develop quantitative assays for:

- Regulated protein expression
- Targeted protein degradation
- Viral infection
- Verification of protein levels from gene expression and RNA-seq experiments
- Protein abundance following pull-downs or transient transfection

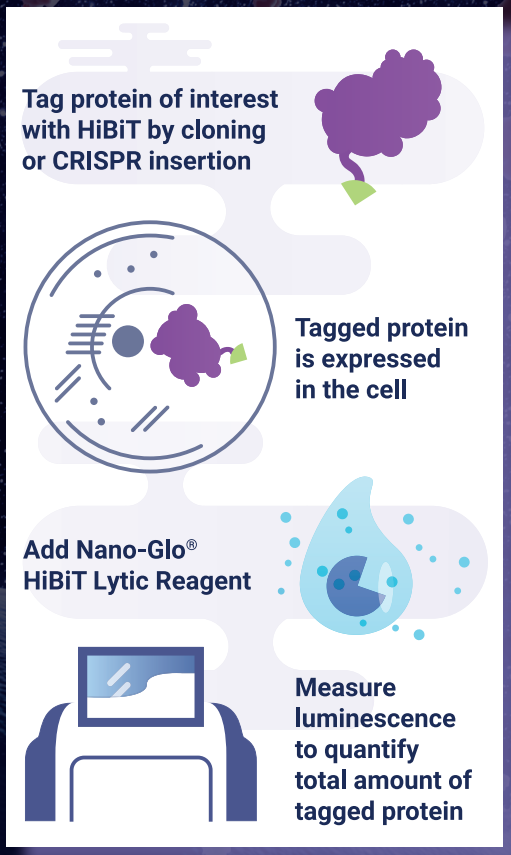
Precise protein quantification

The broad linear dynamic range quantifies tagged proteins regardless of expression level to measure changes in protein abundance. With a limit of detection of less than 10^{-19} moles, the Nano-Glo HiBiT Lytic Detection System can quantify endogenous proteins expressed at low levels.



Nano-Glo® HiBiT Lytic Reagent was added to a titration of purified HaloTag®-HiBiT protein and luminescence measured after 10 minutes.

Overview of the Nano-Glo HiBiT Lytic Detection System Workflow



After the protein of interest has been expressed in the cell, it can be measured by the addition of the Nano-Glo® HiBiT Lytic Reagent on a standard luminometer.

Ordering information

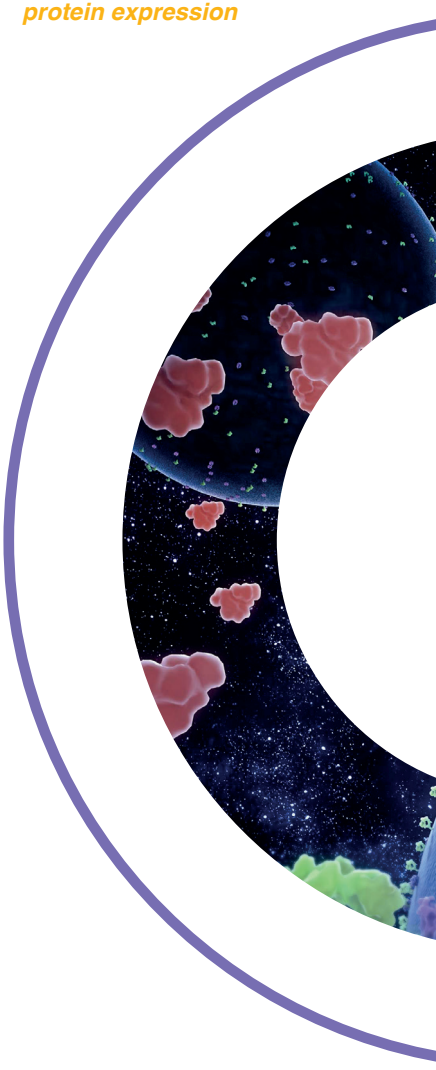
Product	Size	Cat No.
Nano-Glo® HiBiT Lytic Detection System	10ml	N3030
	100ml	N3040
	10 x 100ml	N3050

Nano-Glo HiBiT Extracellular Detection System

Nano-Glo HiBiT Extracellular Detection System quantitates HiBiT-tagged proteins on the cell surface or secreted into the culture medium.

Extracellular HiBiT-tagged protein is measured by adding a non-lytic detection reagent containing the enzyme substrate and LgBiT, which spontaneously interacts with HiBiT to form an active enzyme. The amount of luminescence generated after adding the Nano-Glo HiBiT Extracellular Reagent is proportional to the amount of HiBiT-tagged protein accessible to the medium over seven orders of magnitude.

Simple bioluminescent detection of cell-surface protein expression



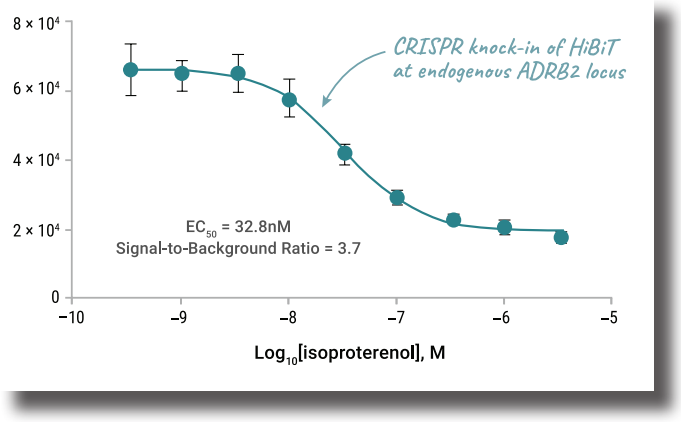
Develop quantitative assays for:

- Receptor internalisation (e.g. G-protein coupled receptors (GPCRs), receptor tyrosine kinases)
- Receptor recycling
- Protein or cytokine secretion
- Surface protein trafficking

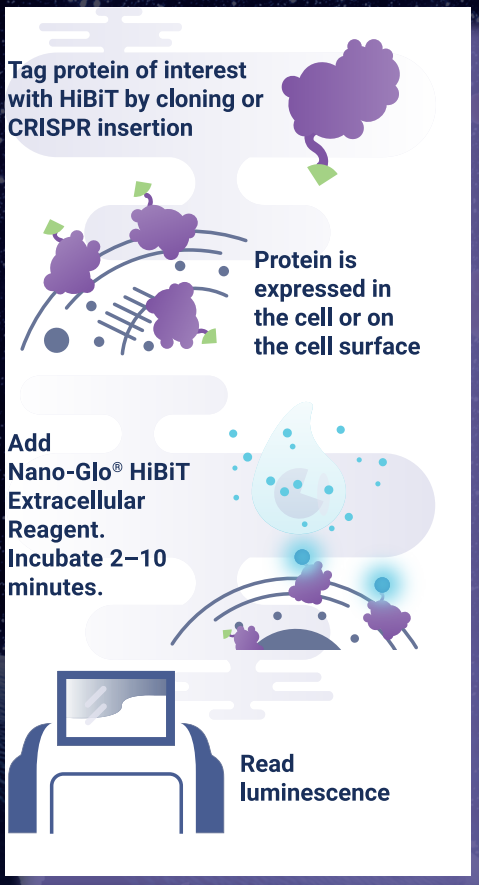
Study receptor activation and internalisation

Nano-Glo HiBiT Extracellular Detection System can quantify activation of receptors, such as GPCRs and EGFR, following treatment with various compounds.

Internalisation of endogenously expressed ADRβ2



HiBiT tag was introduced into cells by CRISPR/Cas9 genome editing. The rates of internalisation of HiBiT-tagged β2 Adrenergic Receptor (ADRβ2) were monitored following treatment with isoproterenol, an ADRβ2 agonist, using the Nano-Glo HiBiT Extracellular Detection System.



After the protein of interest has been expressed on the cell surface, it can be measured by the addition of Nano-Glo HiBitT extracellular reagent.

Ordering information

Product	Size	Cat No.
Nano-Glo® HiBiT Extracellular Detection System	10ml	N2420
	100ml	N2421
	10 x 100ml	N2422

Nano-Glo HiBiT Blotting System

Nano-Glo HiBiT Blotting System is an antibody-free method for characterising HiBiT-tagged proteins separated by SDS-PAGE then transferred to nitrocellulose or PVDF membranes. The amount of HiBiT-tagged protein transferred to the membrane is determined by adding a blotting reagent containing substrate and LgBiT.

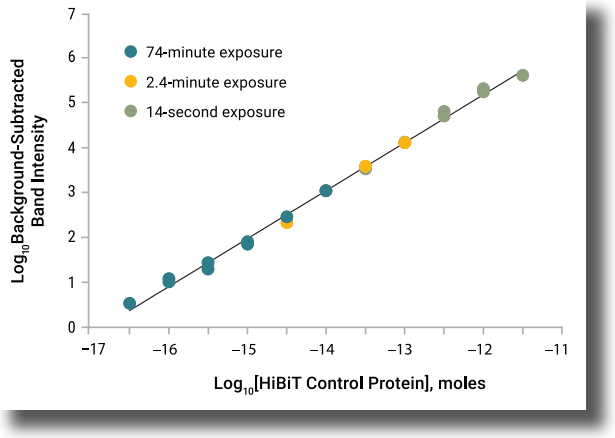
Simple antibody-free detection workflow

Use Nano-Glo HiBiT blotting system to:

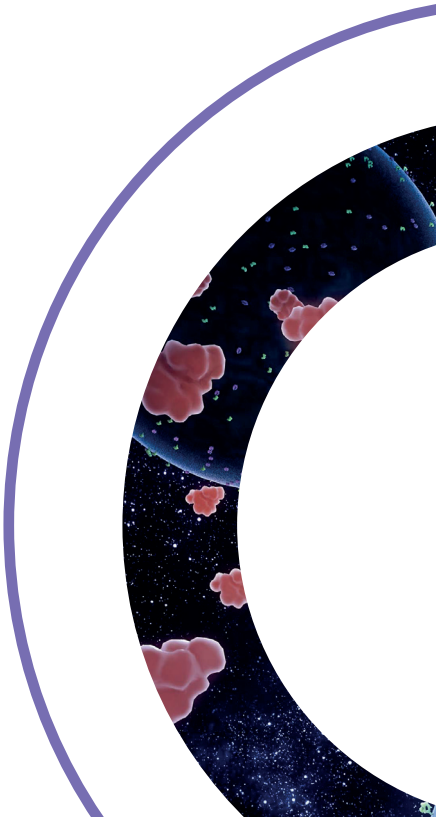
- Determine protein size and quantify expression on blots
- Identify expression of splice variants
- Quickly screen transient transfections or CRISPR knock-ins

Sensitive visualisation of blotted proteins

With its low background and bright signal, the Nano-Glo HiBiT Blotting System can detect small amounts of HiBiT-tagged protein on a membrane (down to femtogram amounts). Luminescence on the blot is proportional to the amount of protein transferred over a range of about five orders of magnitude. This means the system is sensitive enough to detect proteins at endogenous expression levels.

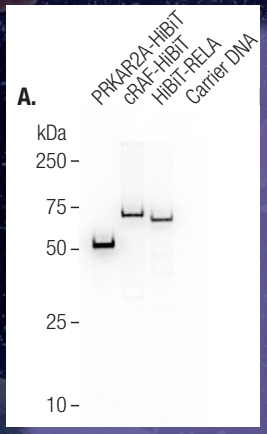


Dynamic range of Nano-Glo® HiBiT Blotting System. Serially diluted HiBiT Control Protein (Cat. No. N3010) was subjected to SDS-PAGE and transferred to a nitrocellulose membrane. Blots were incubated overnight with LgBiT and Nano-Glo® Luciferase Assay Substrate was added before imaging (three different exposure times). A slope of the best-fit line drawn through all of the normalised points indicated proportionality of signal over about five orders of magnitude.



Detect protein size and quantify expression in minutes

HiBiT-tagged proteins present on nitrocellulose membrane can be detected using LgBiT in the presence of substrate. Multiple steps including blocking, antibody binding and washing are simply not required with HiBiT Blotting System. The high affinity interaction between HiBiT-tagged protein and LgBiT generates exceptionally clean blots without background signal.



HiBiT blotting of transiently transfected HeLa cells. HeLa cells transfected with carrier DNA or a CMV-driven expression construct for PRKAR2AHiBiT,cRAF-HiBiT or HiBiT-RELA were subjected to SDS-PAGE and transferred to nitrocellulose membrane. Following incubation in LgBiT/buffer solution (1 hour), Nano-Glo® Luciferase Assay Substrate was added and incubated for 5 minutes and then developed (exposure: 7.4 minutes).

HiBiT Control Protein

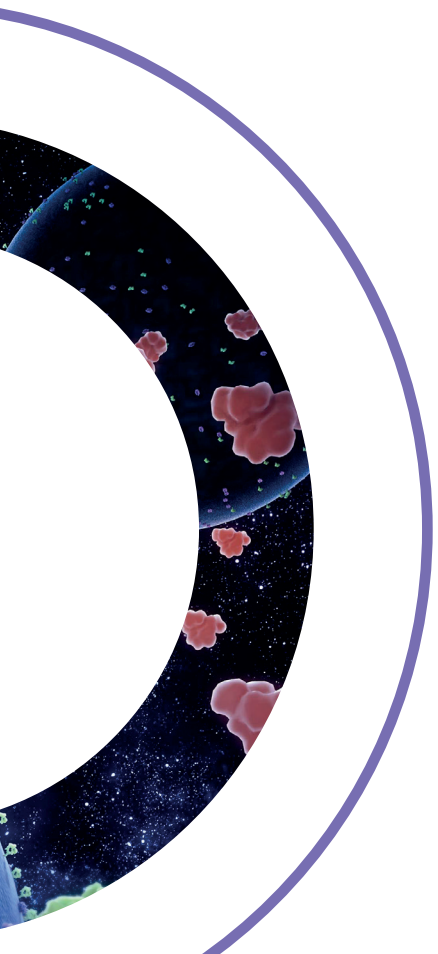
This is a 20µM solution of purified recombinant 36kDa HaloTag® protein fused at its carboxy-terminus to the 11 amino acid HiBiT tag. HiBiT Control Protein can be used as a positive control of known concentration when using the Nano-Glo HiBiT Lytic Detection System, Nano-Glo HiBiT Extracellular Detection System or Nano-Glo HiBiT Blotting System.

Ordering information

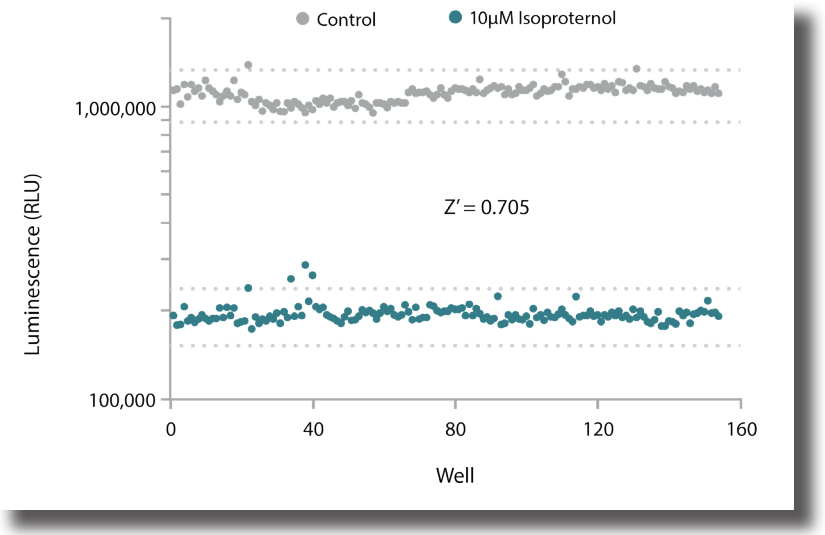
Product	Size	Cat No.
Nano-Glo® HiBiT Blotting System	100ml	N2410
HiBiT Control Protein	100µl	N3010

Scalable from bench to high-throughput screening

Simple protocols and luminescence half-life of greater than 3 hours make Nano-Glo HiBiT Extracellular and Nano-Glo HiBiT Lytic Detection Systems compatible with high-throughput assays. Screening assays can be developed for applications in membrane receptor biology, protein regulation or protein secretion, which are not possible with standard immunodetection methods.



Internalisation of HiBiT-ADRβ2 in stably transfected HEK293 cells in 384-well plates



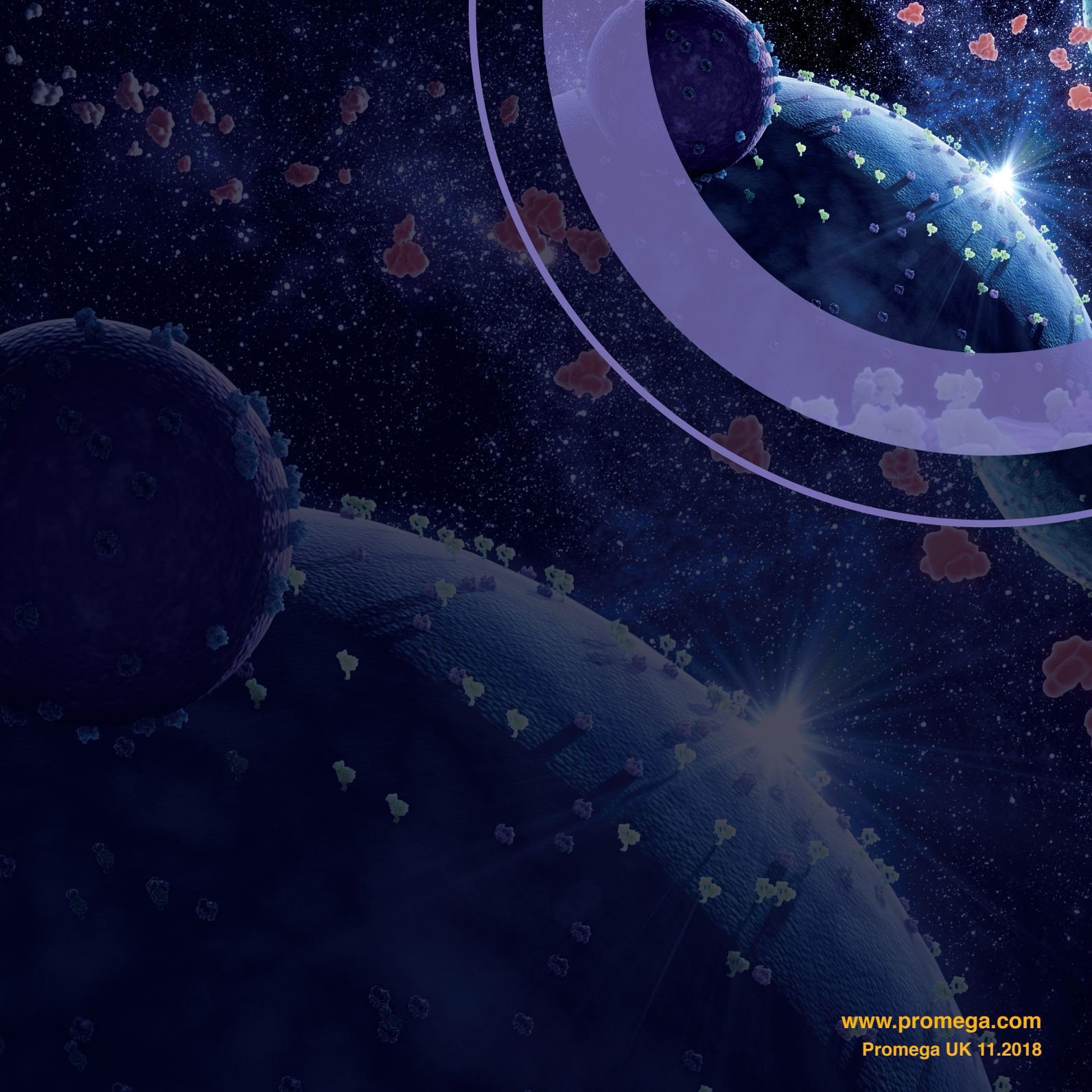
Assay measuring the internalisation of HiBiT-ADRβ2 using the HiBiT Extracellular Detection System

GloMax® Discover, Explorer and Navigator Systems

The highest sensitivity, broadest dynamic range, best value for researchers!

- Plug-n-play assay set-up
- Preloaded protocols
- Integrated with Promega assays
- Automation-friendly for your workflow
- Windows® 8 Tablet PC controlled with state-of the-art touchscreen control and connectivity
- Luminescence, fluorescence, UV-Vis absorbance, BRET, FRET, filtered luminescence available*
- 384 and 96-well plate formats*
- Heating / shaking / injectors*
- Integrated filter paddles for assay multiplexing*
- Part 11 Electronic Signature software compliance
- SiLA-ready software for 3rd party control
- Quick read-speeds for HTS
- IQ/OQ Service

*Model dependent



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