Promega

Explore Your Options for

GPCR Research & Drug Discovery

REG4766

GPCR Research & Drug Discovery

Ligand Binding

Determine direct binding of peptide as well as small molecule ligands to GPCRs using the **NanoBRET™ Target Engagement** assay and the **HiBiT Protein Tagging System**. Create your own assay or benefit from our ready-to-use solutions.

GPCR Interaction

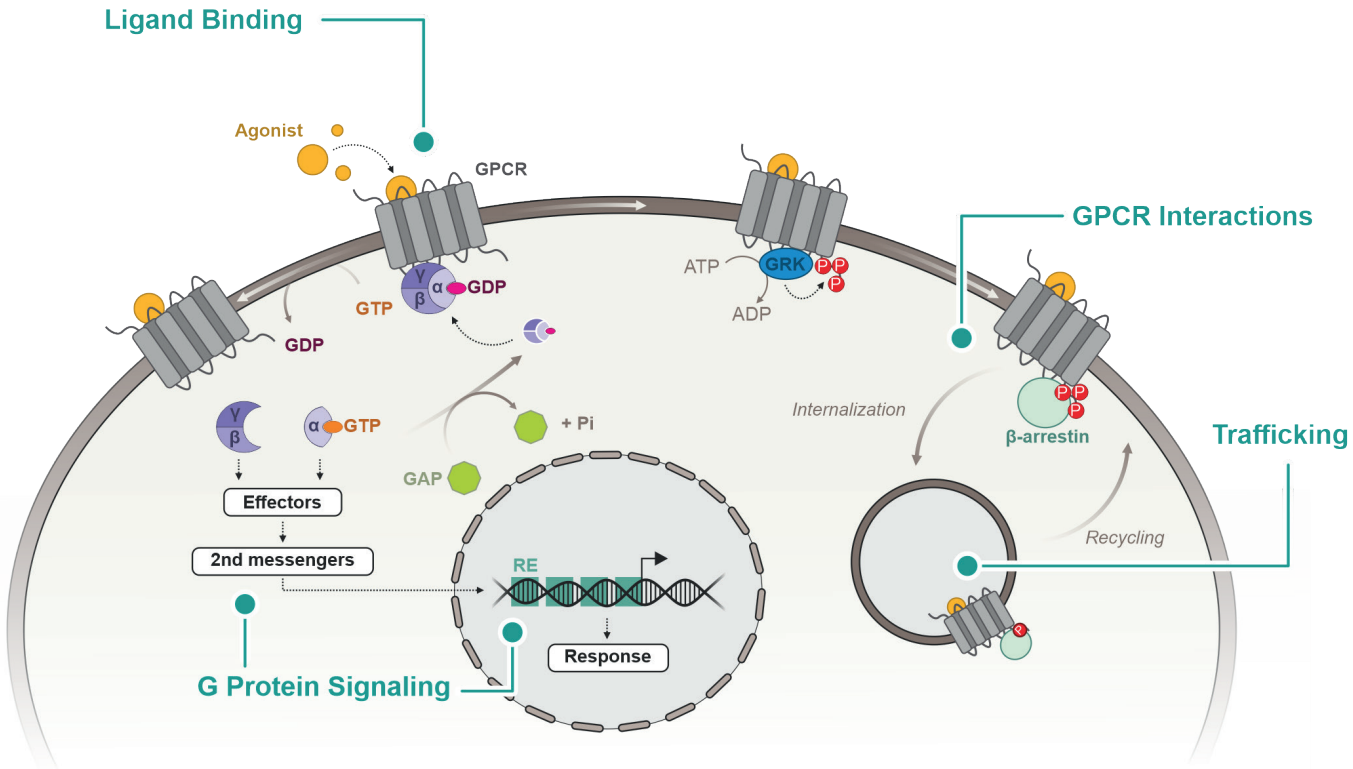
Determine the dynamics of hetero- and homodimeric protein interactions of GPCRs (e.g. β-arrestin recruitment) or interactions within downstream signaling pathways in a live-cell context using the **NanoBiT® and NanoBRET™ Protein:Protein Interaction Systems**.

G Protein Signaling

Investigate GPCR signaling with gene reporter assays using firefly and NanoLuc®-based **Rapid Response™ gene reporter vectors**. Quantify cAMP 2nd messenger levels in either a biochemical enpoint (**cAMP-Glo™ Assay**) or cell-based real-time format (**GloSensor™ cAMP**). Determine downstream phosphorylation events using **Lumit™ Immunoassay Cellular Systems**.

Trafficking

Monitor internalization and recycling processes of GPCRs using the **HiBiT Protein Tagging System**.

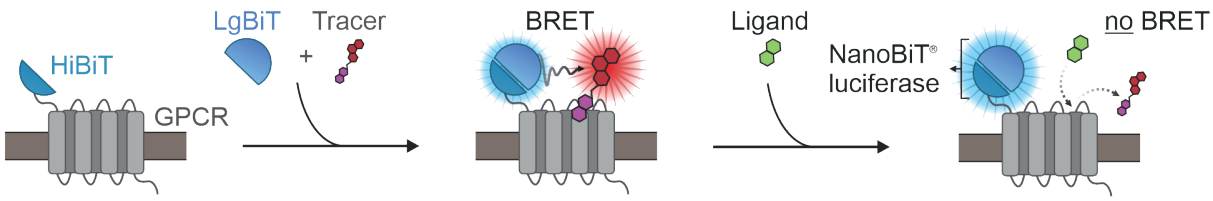


Schematic overview of key processes in GPCR biology.

Product Information

Ligand Binding

Product	Cat. #	Quantity
pBiT3.1-N [CMV/HiBiT/Blast] Vector	N2361	20 µg
pBiT3.1-secN [CMV/HiBiT/Blast] Vector	N2381	20 µg
pFN38K HiBiT CMV-neo Flexi® Vector	N2401	20 µg
pFN39K secHiBiT CMV-neo Flexi® Vector	N2411	20 µg
Please enquire for our large selection of HiBiT-GPCR fusion vectors as well as ready-to-use assays for different GPCR classes.		
Nano-Glo® HiBiT Extracellular Detection System	N2420 / N2421 / N2422	10 / 100 / 10 x 100 ml

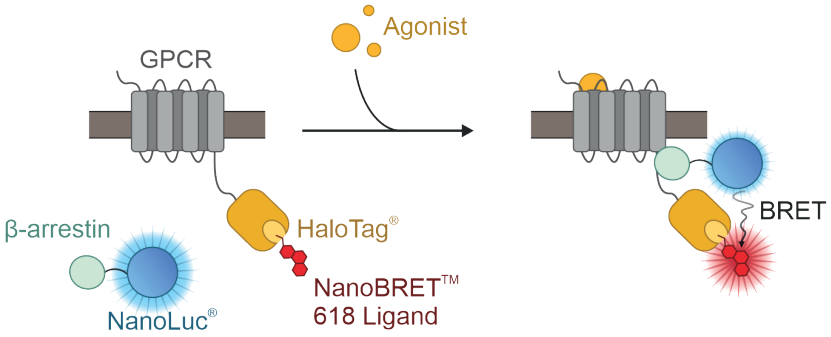


Ligand binding to GPCRs can be quantitatively studied using NanoBRET™ Target Engagement. This assay detects competitive displacement of a fluorescently labeled tracer from a luciferase-GPCR fusion protein. A decrease in BRET signal is indicative of test drug binding. The use of a complemented luciferase donor enables selective analysis of the membrane-bound fraction of receptors. A given GPCR's ectodomain is tagged with the HiBiT peptide and luciferase activity is reconstituted by addition of the membrane-impermeable complementary LgBiT subunit.

GPCR Interaction

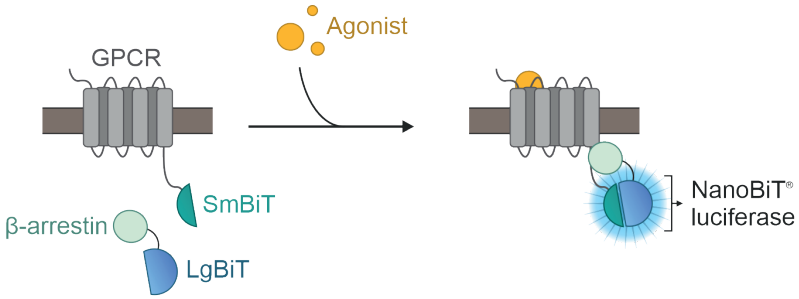
Product	Cat. #	Quantity
NanoBRET™ PPI Starter System Flexi® *or* MCS	N1821 *or* N1811	1 kit
NanoBRET™ Nano-Glo® Detection System	N1661 / N1662 / N1663	200 / 1000 / 10000 assays
NanoBiT® PPI Starter System Flexi® *or* MCS	N2015 *or* N2014	1 kit
NanoBiT® β-arrestin-1 Flexi® Vector Set	CS1603B237 *	1 kit
NanoBiT® β-arrestin-1 MCS Vector Set	CS1603B236 *	1 kit
NanoBiT® β-arrestin-2 Flexi® Vector Set	CS1603B126 *	1 kit
NanoBiT® β-arrestin-2 MCS Vector Set	CS1603B125 *	1 kit
NanoBiT® AVPR2:ARRB2 Control Pair	CS1603B270 *	2 vectors, 20 µg each
NanoBiT® ADRB2:ARRB2 Control Pair	CS1603B57 *	2 vectors, 20 µg each
NanoBiT® CX3CR1:ARRB2 HEK293 Cell Line	CS1603B254 *	2 vials
Nano-Glo® Live-Cell Assay System	N2011 / N2012 / N2013	100 / 1000 / 10000 assays

NanoBRET™ Protein:Protein Interaction



Fusion of the interacting proteins of interest to HaloTag® and NanoLuc® enables analysis of their interaction dynamics while maintaing the live-cell context. Their interaction yields spatial proximity of the luciferase donor and the fluorescently labeled HaloTag® energy acceptor, generating bioluminescence resonance energy transfer (BRET).

NanoBiT® Protein:Protein Interaction



Fusion of the interacting proteins of interest to LgBiT and SmBiT enables analysis of their interaction dynamics while maintaing the live-cell context. Their interaction allows for LgBiT:SmBiT complementation which can be detected by measuring the acitvity of the reconstitued NanoBiT® luciferase.

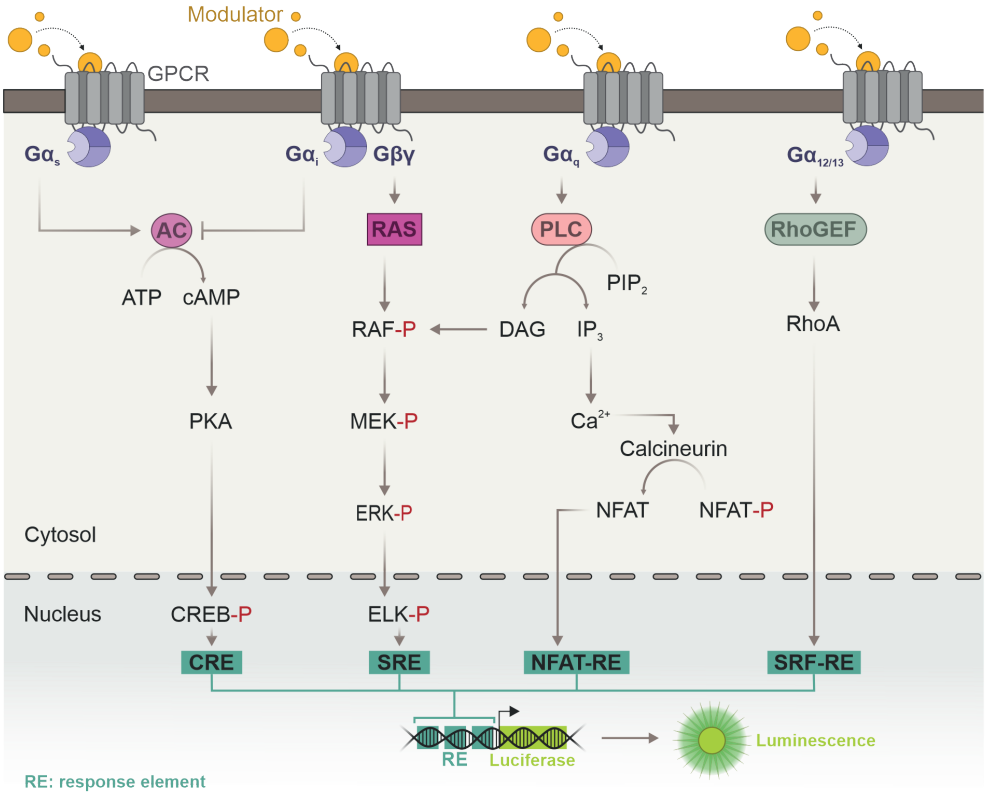
* *This is an Early Access Material. Please enquire for more information.*
For Research Use Only. Not for Use in Diagnostic Procedures.

G Protein Signaling

Transcriptional Activation

Study activity of

Product		Cat. #	Quantity
pNL[*NlucP*/CRE/Hygro]	$G\alpha_s$	CS186804 *	20 µg
pNL[*NlucP*/SRE/Hygro]	$G\alpha_i$	CS177601 *	20 µg
pNL[*NlucP*/NFAT-RE/Hygro]	$G\alpha_q$	CS177602 *	20 µg
pNL[*NlucP*/SRF/Hygro]	$G\alpha_{12}$	CS194101 *	20 µg
Nano-Glo® Luciferase Assay System		N1110 / N1120 / N1130	10 / 100 / 10 x 10 ml
pGL4.29[*luc2P*/CRE/Hygro]	$G\alpha_s$	E8471	20 µg
pGL4.33[*luc2P*/SRE/Hygro]	$G\alpha_i$	E1340	20 µg
pGL4.30[*luc2P*/NFAT-RE/Hygro]	$G\alpha_q$	E8481	20 µg
pGL4.34[*luc2P*/SRF-RE/Hygro]	$G\alpha_{12}$	E1350	20 µg
ONE-Glo™ Luciferase Assay System		E8110 / E8120 / E8130	10 / 100 / 10 x 10 ml

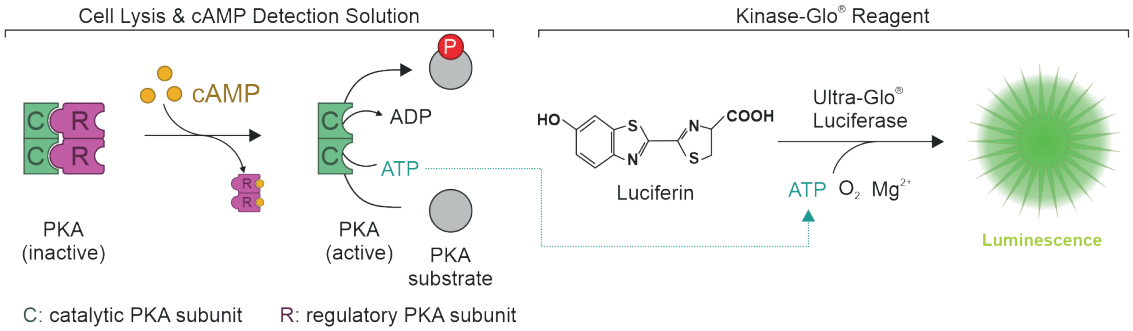


Overview of GPCR signaling pathways and their analysis using Luciferase-based gene reporter assays.

Check out the Nano-Glo® Dual-Luciferase® Reporter Assay System
www.promega.com/TryDLR

Second Messenger Analysis in Cell Lysates

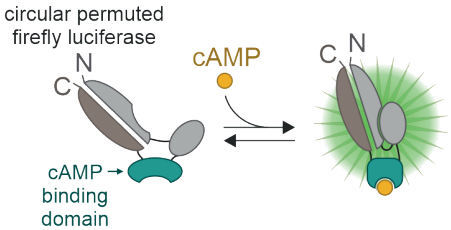
Product	Cat. #	Quantity
cAMP-Glo™ Assay	V1501 / V1502	300 / 3000 assays
cAMP-Glo™ Max Assay	V1681 / V1682 / V1683	2 / 20 / 10 x 20 plates



The cells are induced with a test compound for an appropriate period of time to modulate cAMP levels. Cells are then lysed, and released cAMP is measured by its ability to stimulate the catalytic activity of protein kinase A (PKA). Kinase-Glo® Reagent is then added to terminate the PKA reaction and quantify remaining ATP via a luciferase reaction.

Second Messenger Analysis in Live Cells

Product	Cat. #	Quantity
pGloSensor™-20F cAMP Plasmid	E1171	20 µg
pGloSensor™-22F cAMP Plasmid	E2301	20 µg
GloSensor™ cAMP 22F HEK293 Cell Line	CS305302 *	2 vials
pGloSensor™-22F cAMP/Aequorin HEK293 Cell Line	CS174801 *	2 vials
GloSensor™ cAMP Reagent	E1290 / E1291	25 / 250 mg
Coelentrazine	S2001	250 µg



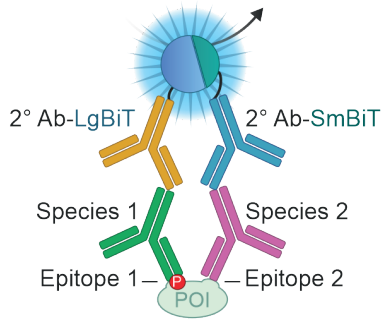
The GloSensor™ cAMP assay uses a firefly luciferase-based biosensor that enables monitoring of intracellular cAMP in a live-cell assay format. This biosensor consists of a circularly permuted form of firefly luciferase that is fused to cAMP binding domains with a range of affinities for cAMP. Analyte binding leads to a conformational change that promotes large increases in luminescence activity. The magnitude of the luminescence increase is directly proportional to the amount of analyte present.

** This is an Early Access Material. Please enquire for more information.*
For Research Use Only. Not for Use in Diagnostic Procedures.

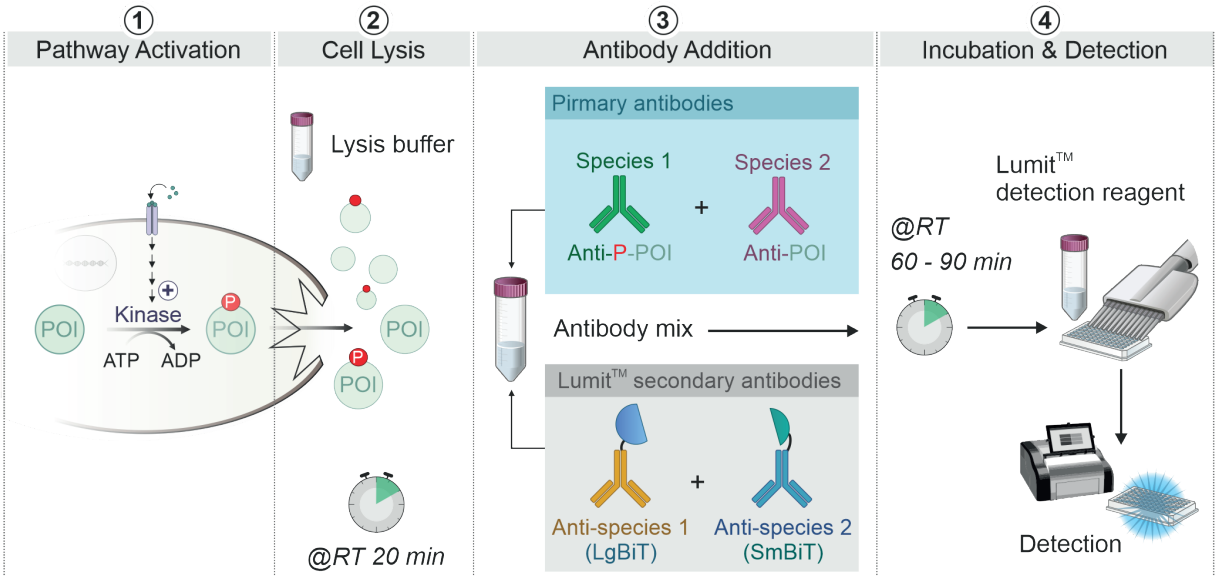
Protein Phosphorylation

Application Notes available for phospho-CREB, -ERK...

Product	Cat. #	Quantity
Lumit™ Immunoassay Cellular Systems Starter Kit	W1220	1 kit
Lumit™ Immunoassay Cellular Systems – Set 1	W1201 / W1202 / W1203	100 / 1000 / 10000 assays
Lumit™ Immunoassay Cellular Systems – Set 2	W1331 / W1332 / W1333	100 / 1000 / 10000 assays
Lumit™ Immunoassay Lysis and Detection Kit	W1231 / W1232 / W1233	100 / 1000 / 10000 assays
Lumit™ Anti-Mouse Ab-LgBiT	W1021 / W1022	30 / 300 µl
Lumit™ Anti-Mouse Ab-SmBiT	W1051 / W1052	30 / 300 µl
Lumit™ Anti-Rabbit Ab-LgBiT	W1041 / W1042	30 / 300 µl
Lumit™ Anti-Rabbit Ab-SmBiT	W1031 / W1032	30 / 300 µl
Lumit™ Anti-Goat Ab-LgBiT	W1061 / W1062	30 / 300 µl
Lumit™ Anti-Goat Ab-SmBiT	W1071 / W1072	30 / 300 µl



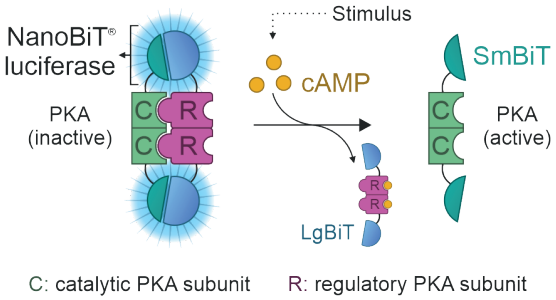
Lumit™ Immunoassays Cellular Systems are based on the NanoBiT® complementation reporter. These assays enable fast, convenient, and sensitive quantification of phosphoproteins within cell lysates.



The experimental workflow starts with **(1)** the treatment of cells to activate the signaling pathway of interest. **(2)** Cells are lysed in-well by addition of a digitonin-based lysis buffer. **(3)** Following addition of the antibody mix (primary and Lumit™ secondary antibodies) and **(4)** incubation for 60 to 90 minutes, the luminescent signal of the assay is determined by addition of the Lumit™ detection reagent.

Protein Interactions

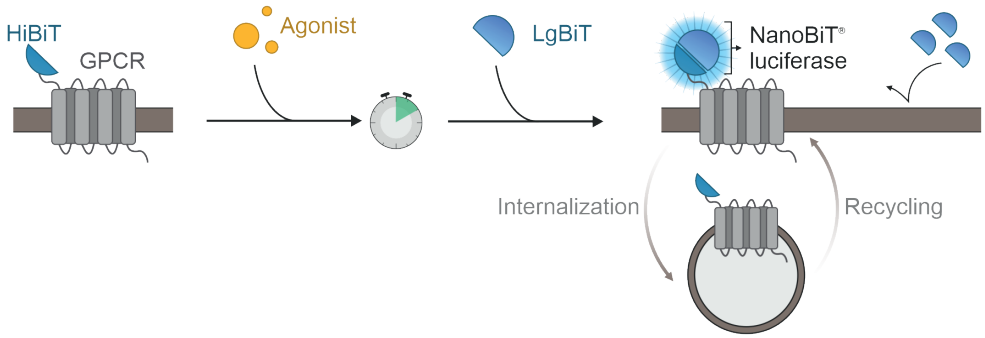
Product		Cat. #	Quantity
NanoBiT® Control Pair – PRKACA/PRKAR2A	TK promoter	CS1603B47 *	2 vectors, 20 µg each
Please enquire for a CMV-based version of these vectors.			



Protein kinase A (PKA) is an important downstream effector of GPCRs that integrate their signals through modulation of cAMP levels. Its activation status can be monitored in live cells using the NanoBiT® Protein:Protein Interaction System in order to identify and characterize modulators of $G\alpha_s$ and $G\alpha_i$ protein coupled receptors.

Trafficking

Product	Cat. #	Quantity
pBiT3.1-N [CMV/HiBiT/Blast] Vector	N2361	20 µg
pBiT3.1-secN [CMV/HiBiT/Blast] Vector	N2381	20 µg
pFN38K HiBiT CMV-neo Flexi® Vector	N2401	20 µg
pFN39K secHiBiT CMV-neo Flexi® Vector *Please enquire for a CMV-based version of these vectors.*	N2411	20 µg
Nano-Glo® HiBiT Extracellular Detection System	N2420 / N2421 / N2422	10 / 100 / 10 x 100 ml
Nano-Glo® Vivazine™ Substrate	N2580 / N2581 / N2582	0.1 / 1 / 10 ml



Receptor trafficking upon agonist stimulation is a central aspect of GPCR biology. This dynamic process can be studied using the HiBiT Protein Tagging System. Because the LgBiT subunit is membrane-impermeable, only surface receptors are quantified, so receptor internalization leads to a loss of signal. Real-time formats are also possible for monitoring the kinetics of internalization and recycling.

** This is an Early Access Material. Please enquire for more information.*
For Research Use Only. Not for Use in Diagnostic Procedures.

References

Ligand Binding

» Schihada, H. *et al.* (2020) *Development of a conformational histamine H3 receptor biosensor for the synchronous screening of agonists and inverse agonists.* ACS Sens. 5(6):1734–1742

» Killoran, MP. *et al.* (2020) *An Integrated Approach toward NanoBRET Tracers for Analysis of GPCR Ligand Engagement.* Molecules. 26(10):12857

» Boursier, ME. *et al.* (2020) *The luminescent HiBiT peptide enables selective quantitation of GPCR ligand engagement and internalization in living cells.* J Biol Chem. (15):5124–5135

» White, CW. *et al.* (2019) *NanoBRET ligand binding at a GPCR under endogenous promotion facilitated by CRISPR/Cas9 genome editing.* Cell Signal. 54:27–34

» Stoddart LA. *et al.* (2018) *NanoBRET Approaches to Study Ligand Binding to GPCRs and RTKs.* Trends Pharmacol Sci. 39(2):136–147

» Alcobia, DC. *et al.* (2018) *Visualizing Ligand Binding to a GPCR In Vivo Using NanoBRET.* iScience. 6:280–288

GPCR Interaction

» Wouters, E. *et al.* (2019) *Distinct dopamine D2 receptor antagonists differentially impact D2 receptor oligomerization.* Int J Mol Sci. 20(7):1686

» Littmann, T. *et al.* (2019) *Split luciferase-based assay for simultaneous analyses of the ligand concentration- and time-dependent recruitment of β-arrestin2.* Anal Biochem. 573:8–16

» Habara, M. *et al.*(2018) *Molecular characterization of feline melanocortin 4 receptor and melanocortin 2 receptor accessory protein 2.* Gen. Comp. Endocrinol. 261, 31–9.

» White, CW. *et al.* (2017) *Using NanoBRET and CRISPR/Cas9 to monitor proximity to a genome edited protein in real-time.* Sci. Reports 7, 3187

» Dixon, AS. *et al.* (2016) *NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells.* ACS Chem Biol. 11(2):400–8

» Machleidt, T. *et al.* (2015) *NanoBRET – A Novel BRET Platform for the Analysis of Protein:Protein Interactions.* ACS Chem Biol. 10(8):1797–804

G Protein Signaling

» Swiatnicki, M. *et al.* (2022) *Profiling oncogenic KRAS mutant drugs with a cell-based Lumit p-ERK immunoassay.* SLAS Discov. 27(4):249–257

» Hwang, B. *et al.* (2020) *A homogeneous bioluminescent immunoassay to probe cellular signaling pathway regulation.* Commun Biol. 3:8

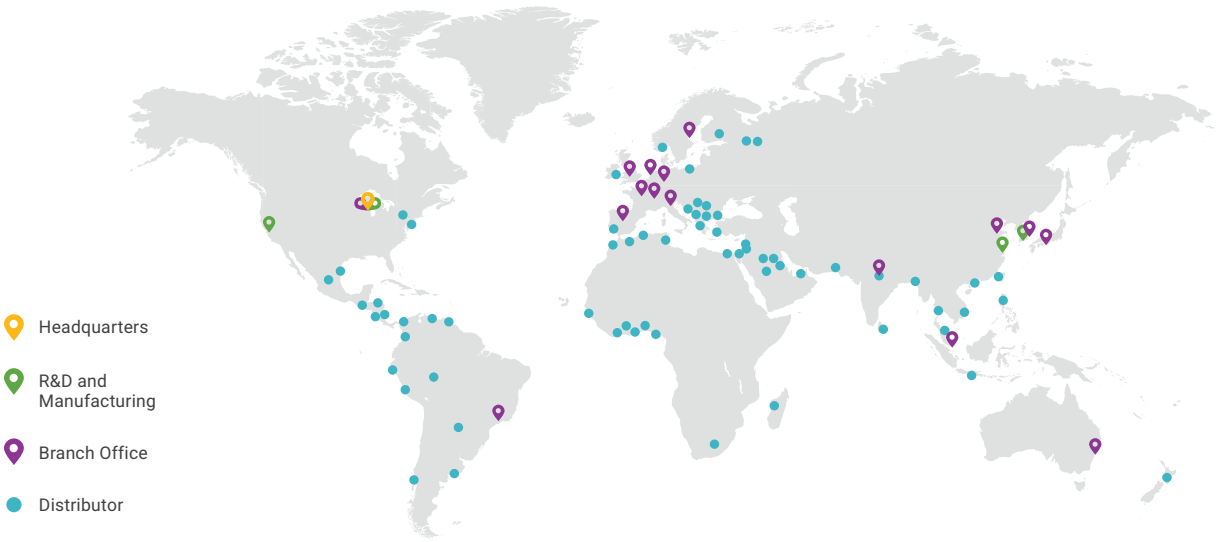
» Cheng, Z. *et al.* (2010) *Luciferase Reporter Assay System for Deciphering GPCR Pathways.* Curr Chem Genomics. 4:84–91.

» Kumar, M. *et al.* (2007) *A bioluminescent-based, HTS-compatible assay to monitor G-protein-coupled receptor modulation of cellular cyclic AMP.* Assay Drug Dev Technol. 5(2)237–45

Trafficking

» Rouault, AAJ. *et al.* (2017) *Regions of MRAP2 required for the inhibition of orexin and prokinecticin receptor signaling.* Biochim. Biophys. Acta Mol. Cell. Res. 1864, 2322–9.

» Soave, M. *et al.* (2020) *NanoBiT Complementation to Monitor Agonist-Induced Adenosine A1 Receptor Internalization.* SLAS Discov. 25(2):186–194



EUROPE

Germany/Austria
Tel: +49 6227 6906 0
Fax: +49 6227 6906 222
E-mail: de_custserv@promega.com

Switzerland
Tel: +44 878 90 00
Fax: +44 878 90 10
E-mail: ch_custserv@promega.com

France
Tel: +33 0437 2250 00
Fax: +33 0437 2250 10
Numero Vert: 0 800 48 79 99
E-mail: contactfr@promega.com

Italy
Tel: +39 0254 0501 94
Fax: +39 0256 5616 45
Toll-Free Phone: 800 6918 18
E-mail: customerservice.italia@promega.com

Poland
Tel: +48 22 531 0667
Fax: +48 22 531 0669
E-mail: pl_custserv@promega.com

Belgium/Luxembourg/The Netherlands
Tel: +31 71 532 42 44
Fax: +31 71 532 49 07
E-mail: benelux@promega.com

Spain
Tel: +34 902 538 200
Fax: +34 902 538 300
E-mail: esp_custserv@promega.com

Denmark, Estonia, Finland, Iceland, Norway, Sweden
Tel: +46 8 452 2450
Fax: +46 8 452 2455
E-mail: sweorder@promega.com

United Kingdom
Tel: +44 23 8076 0225
Fax: +44 23 8076 7014
Free Phone: 0800 378994
E-mail: ukcustserve@promega.com

NORTH AMERICA

United States/Canada
Tel: +1 608 274 4330
Fax: +1 608 277 2516
Toll-Free Tel: 800 356 9526
Toll-Free Fax: 800 356 1970
E-mail: custserv@promega.com

SOUTH AMERICA

Brazil
Tel: +55 11 5090 3780
Fax: +55 11 5096 3780
E-mail: promega.brasil@promega.com

ASIA

China
Tel: +86 10 5825 6268
Fax: +86 10 5825 6160
Toll-Free: 800 810 8133
E-mail: info@promega.com.cn

Japan
Tel: +81 3 3669 7981
Fax: +81 3 3669 7982
E-mail: jpmktg@jp.promega.com

Korea
Tel: +82 2 1588 3718
Fax: +82 2 2638 5418
E-mail: CustServiceKR@promega.com

Pacific Asia Region, Singapore
Tel: +65 6513 3450
Fax: +65 6773 5210
E-mail: sg_custserv@promega.com

India
Tel: +91 11 43005814/15/16/17
Fax: +91 11 41035028
E-mail: ind_custserv@promega.com

AUSTRALIA

Australia
Tel: +61 2 8338 3800
Fax: +61 2 8338 3855
Freecall: 1800 225123
Freefax: 1800 626 017
E-mail: auscustserv@promega.com

For more information about Promega Branch Offices and Distributors visit:
www.promega.com/support/worldwide-contacts