GloSensor™ Technology: Overview

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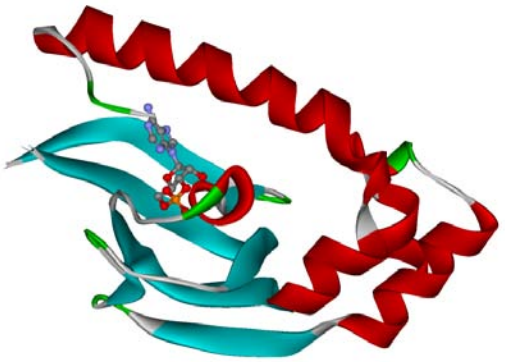
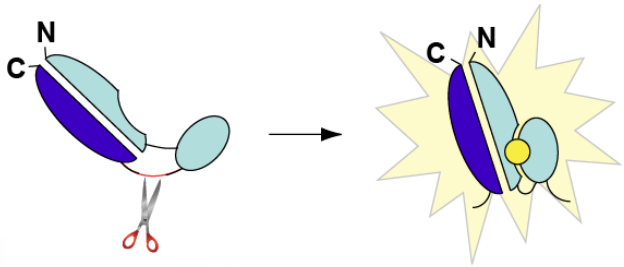
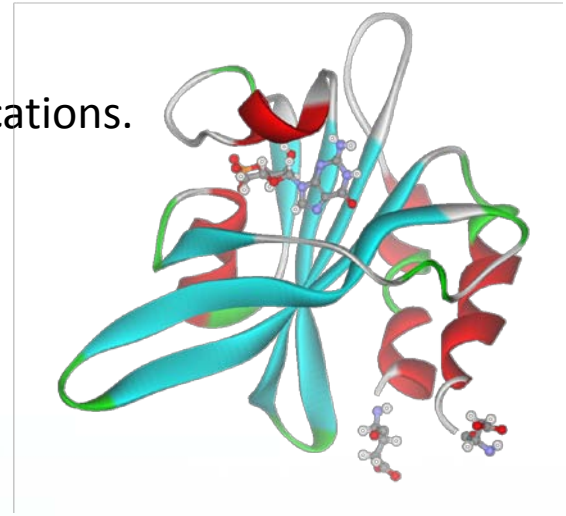
GloSensor™ Technology

GloSensor™ represents a platform technology of biosensors for the intracellular detection of signal transduction in living cells. Promega scientists developed the intracellular biosensor concept using directed evolution resulting in a suite of novel constructs that are easier to use and more sensitive than any other product on the market.



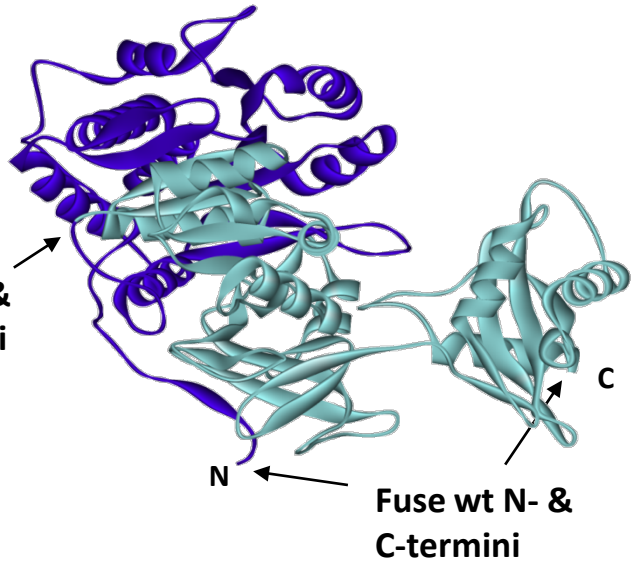
This slide decks offers a brief overview of the GloSensor™ technology with sample data and additional resources including peer-review publications.



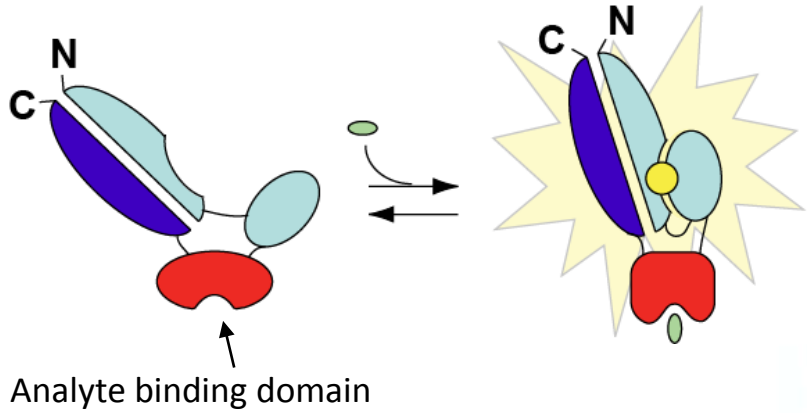


GloSensor™ Technology (con't.)

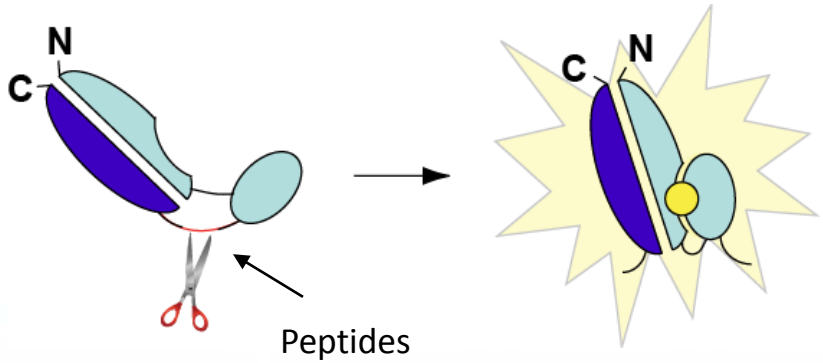
Luminescent biosensors are created via fusion & circular permutation of luciferase



cAMP & cGMP Detection:



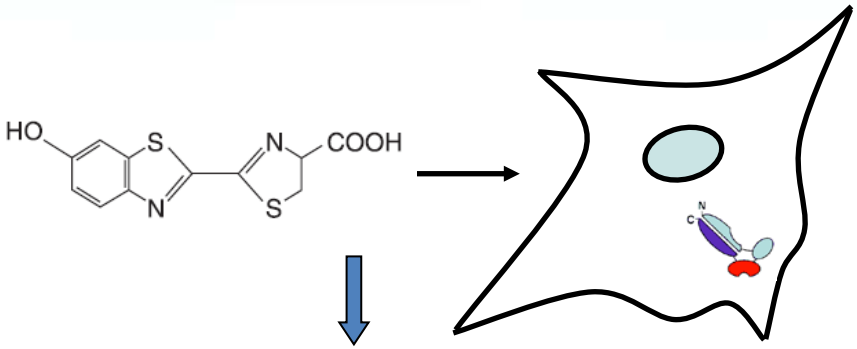
Protease Detection:



We have a number of custom GloSensor™ materials, including plasmids, vectors & cell lines for sale. Please send inquiries to: CAS@promega.com

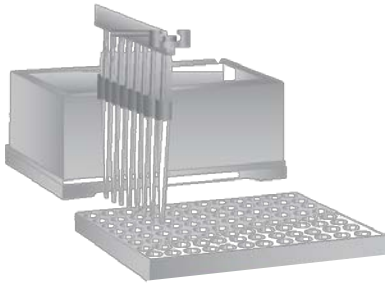
Live Cell, Non-Lytic Assay Format

1. Pre-equilibrate w/ substrate



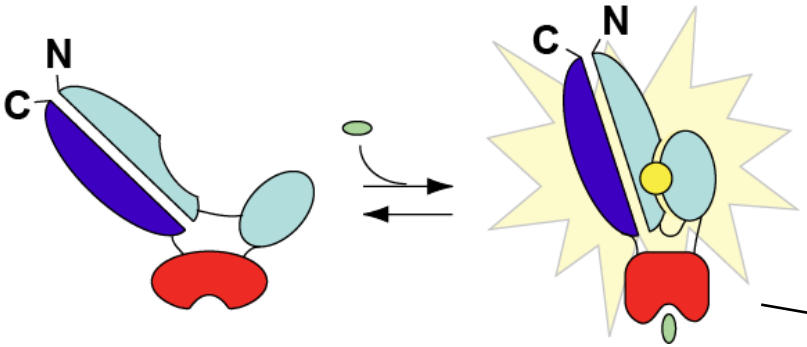
Transient or stable biosensor expression

2. Mix compounds & cells

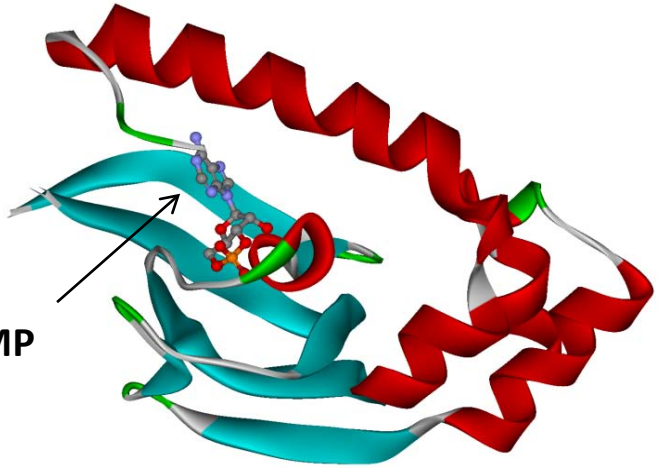


3. Kinetic or end-point measurements

GloSensor™ cAMP Assay



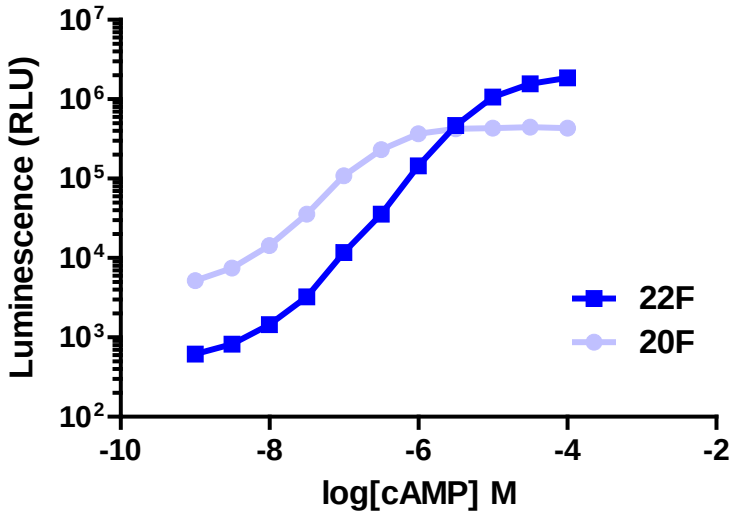
Human RIIβB structure

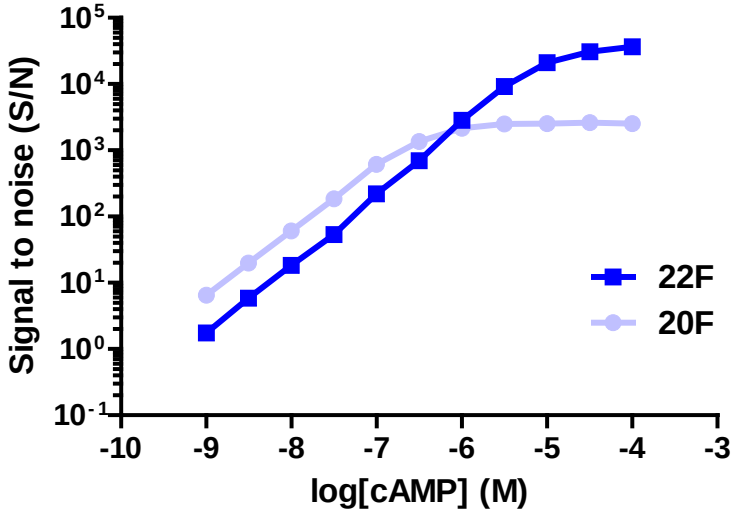


<u>Analyte binding domain:</u>
cAMP binding domain B from human PKA regulatory subunit type IIβ

GloSensor™ cAMP Variants In Vitro

pGloSensor™-20F cAMP Plasmid (Cat.# E1171) & **pGloSensor™-22F cAMP Plasmid** (Cat.# E2301)

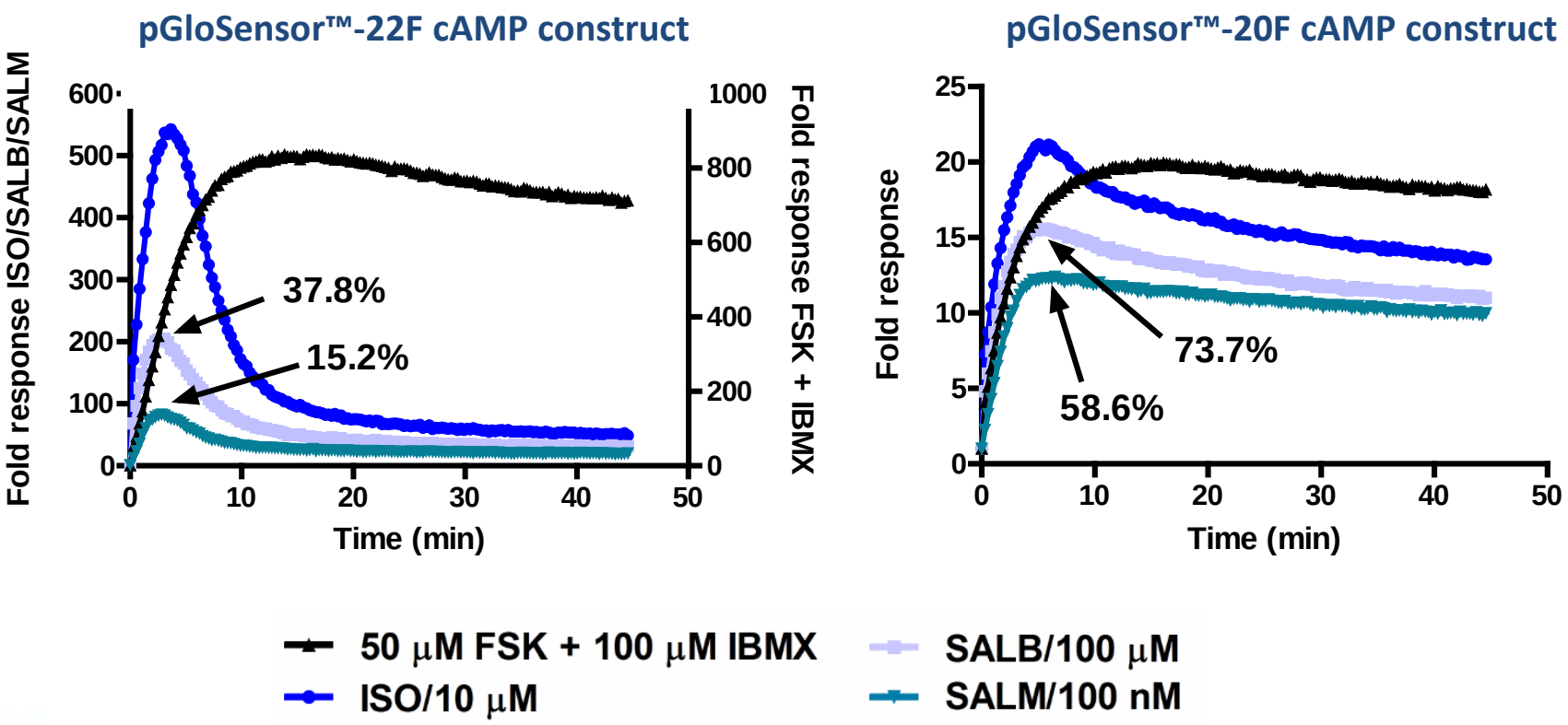




	20F	22F
EC_{50} (µM)	0.3	9.0
Detection range	0.003-10µM	0.003-100µM

Cell-free expression using TNT® SP6 Coupled Reticulocyte Lysate System (Cat.# L4610), n = 3 per dose

GloSensor™ cAMP Variants in HEK293 Cells



Transient expression in HEK293 cells, n = 1 per trace

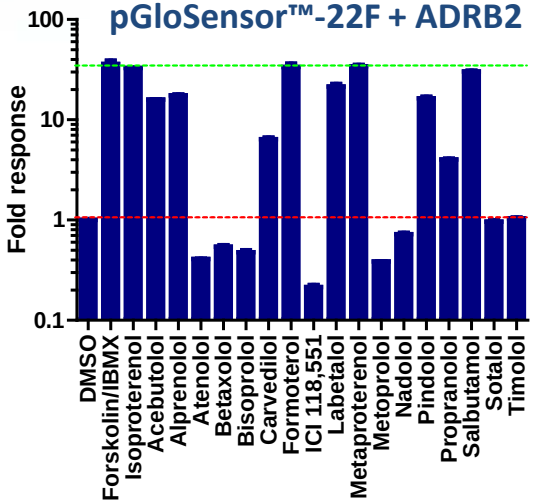
Advantages of Construct ‘22F’ Over ‘20F’

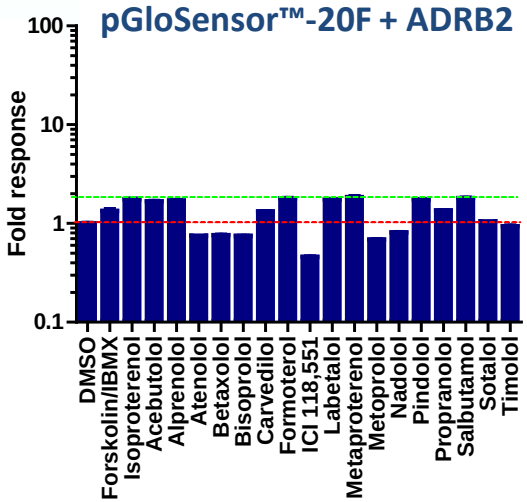
In our hands, we have seen multiple advantages in using pGloSensor™-22F cAMP Plasmid over pGloSensor™-20F cAMP Plasmid:

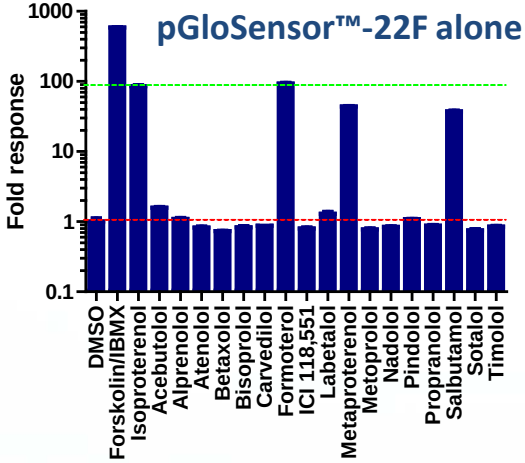
1. 10-30 fold increase in S/B for endogenous or overexpressed Gs-coupled receptors
2. 2-4 fold increase in S/B for overexpressed Gi-coupled receptors
3. Reduced tendency to approach saturation in living cells
4. Improved performance for endogenous Gs-coupled receptors in non-HEK293 cell types

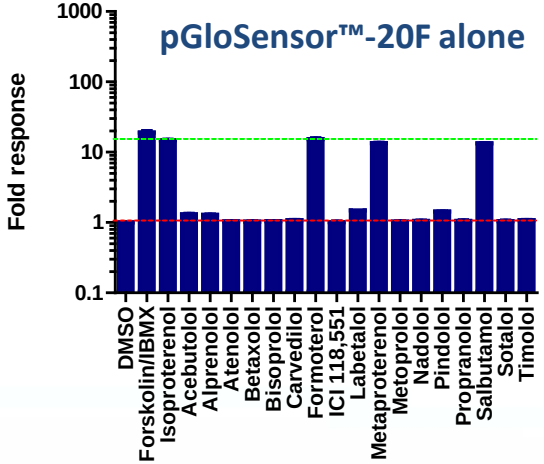
For interest in custom GloSensor™ materials, including plasmids, vectors & cell lines, please send inquiries to: CAS@promega.com

Full, Partial & Inverse Agonist Detection







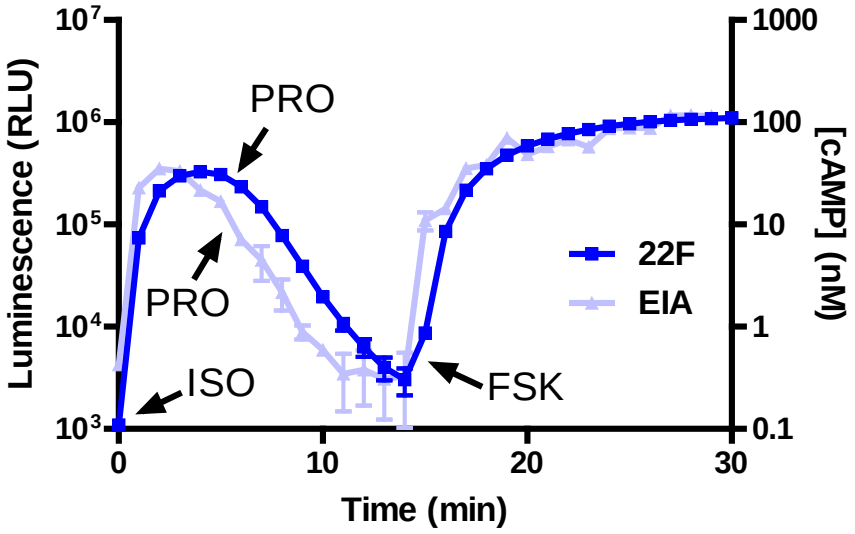


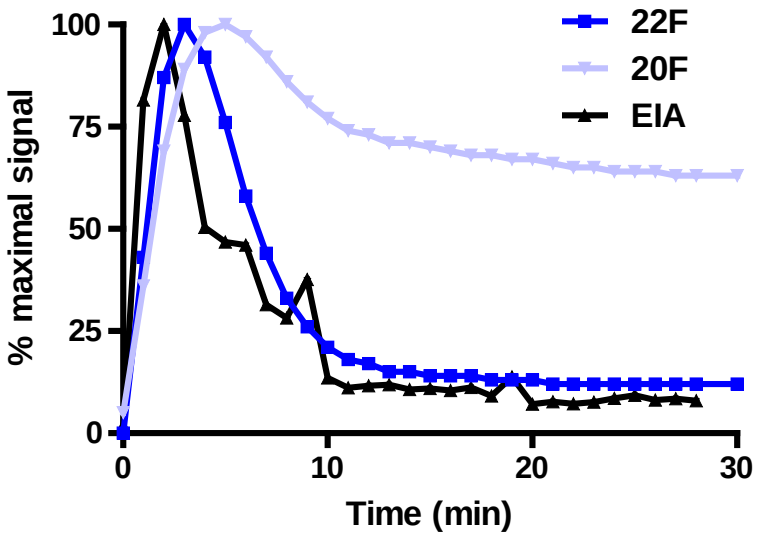
Full, partial and inverse agonist detection in the same experiment

Transient expression in HEK293 cells; 10µM compounds; n = 3

GloSensor™ cAMP Assay vs. an Immunoassay

Predictive, real-time detection of intracellular [cAMP]

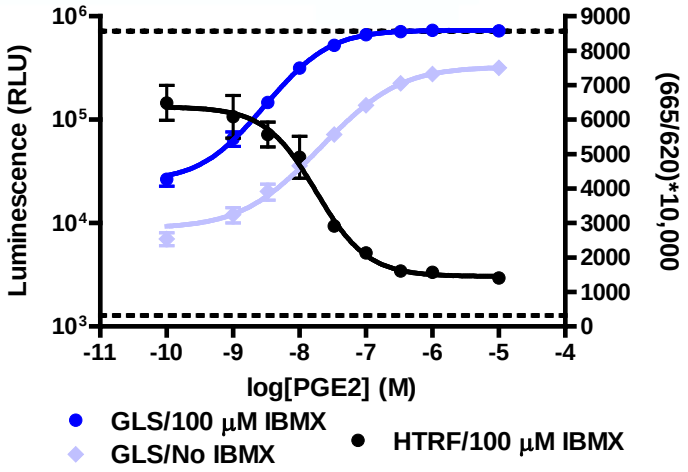


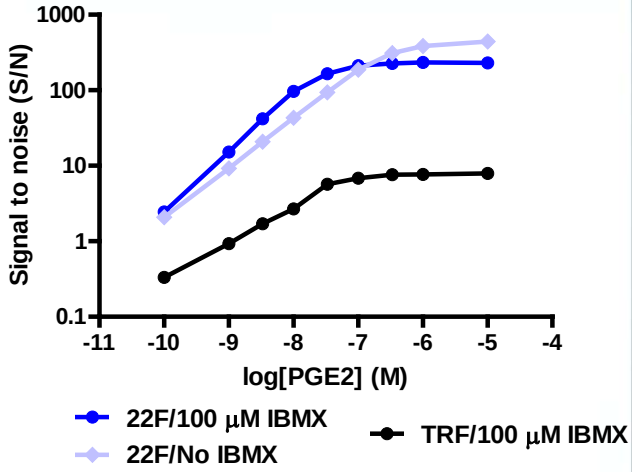


Immunoassay by Assay Designs (Cat. # 901-066, acetylated); n = 3 per data point

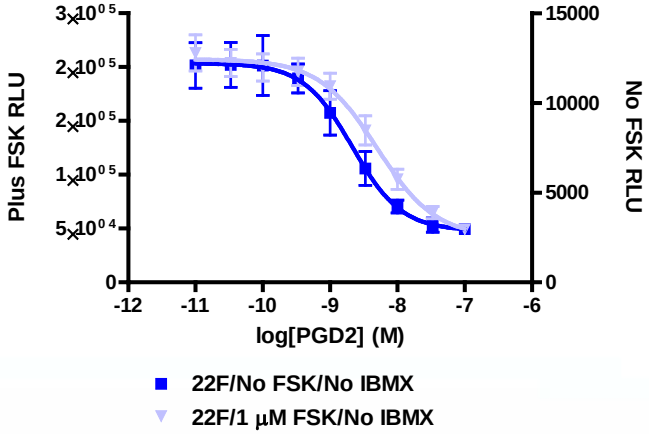
GloSensor™ cAMP vs. Time-resolved Fluorescence

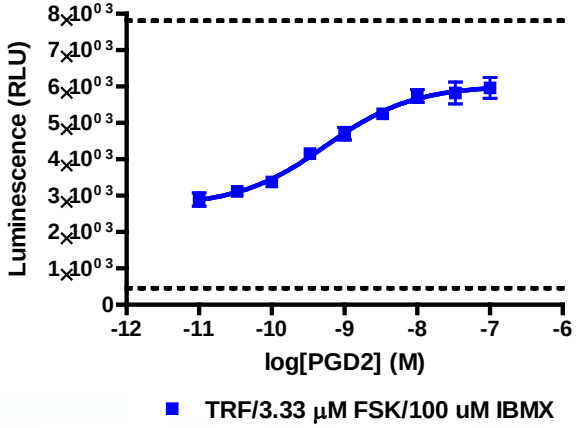
Endogenous EP receptor (Gs-coupled)





Overexpressed DP2 receptor (Gi-coupled)

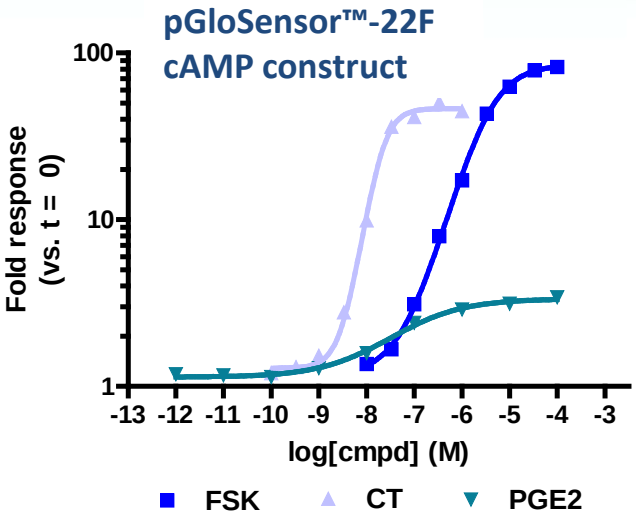


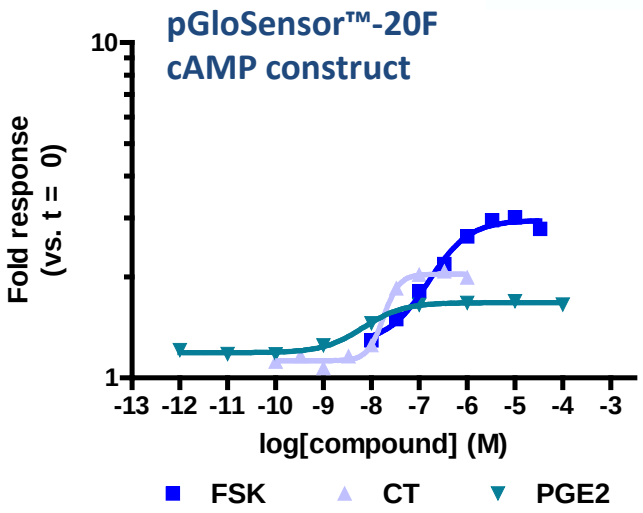


Cisbio cAMP kit - 384-well assay format (20 µl), cAMP HiRange, Cat.# 62AM6PEB

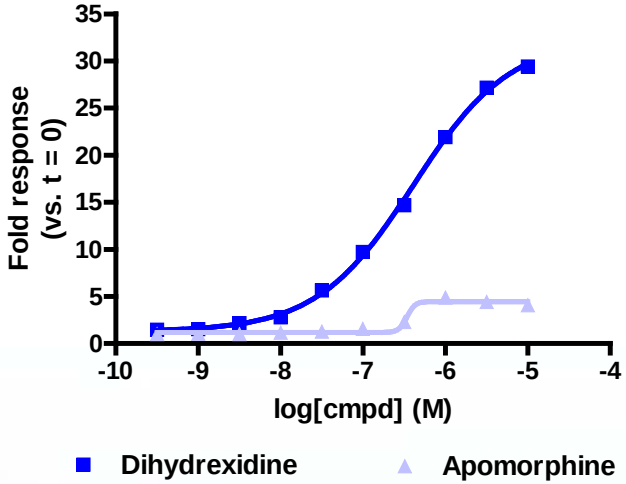
GloSensor™ cAMP Assay in CHO & U2OS Cells

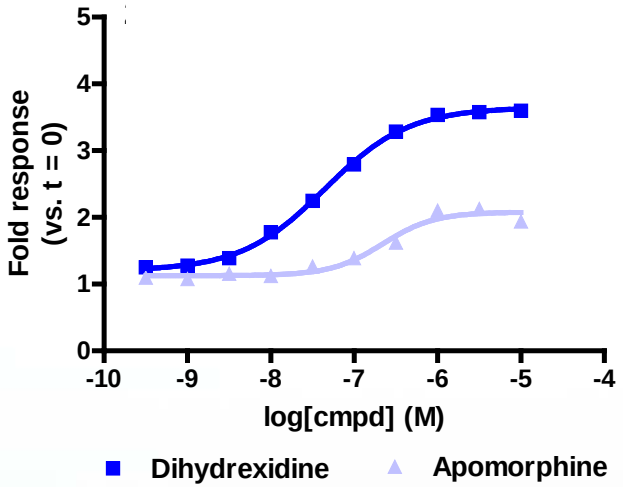
CHO cell background



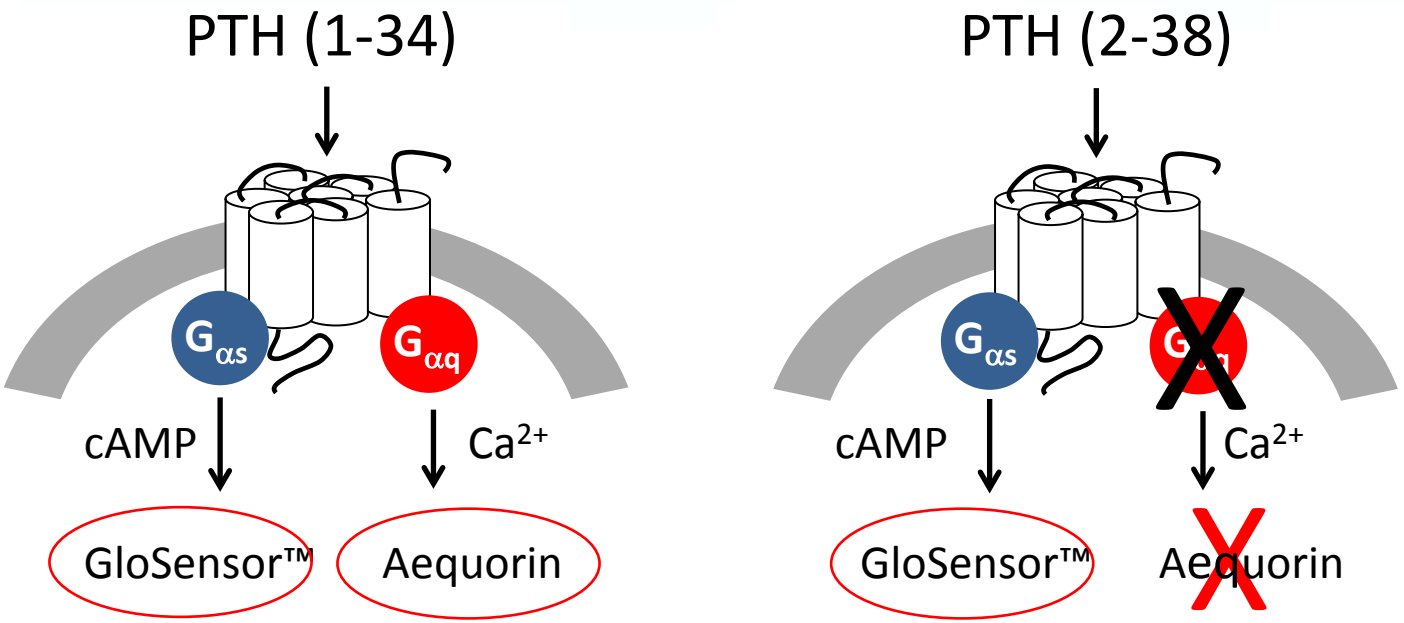


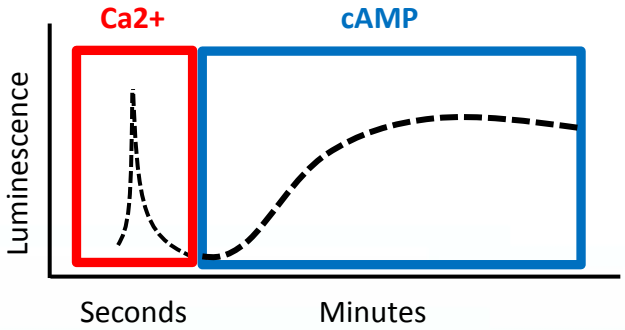
U2OS cell background





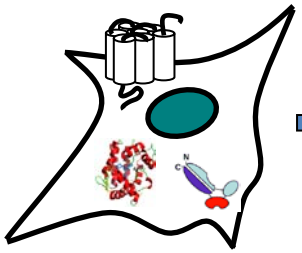
Measuring Two Second Messenger Pathways in Real-time





GloSensor™ cAMP Assay + Aequorin: Multiplex Workflow

Day 1: Transient transfection:
- PTH1R
- GloSensor™ cAMP Assay
- Aequorin



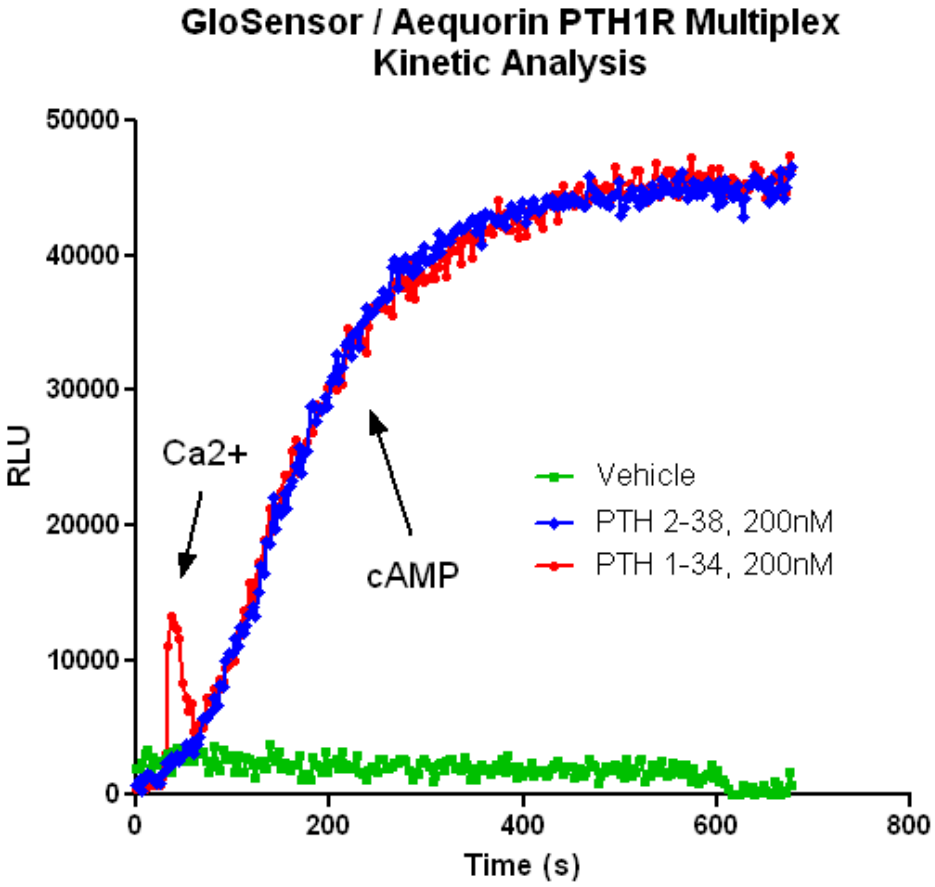
Day 2: Incubate cells with substrates concurrently (2hr ambient temperature)

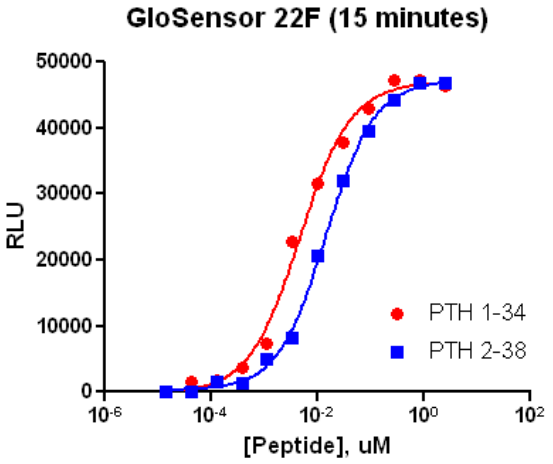
GloSensor™ Reagent + Aequorin Substrate

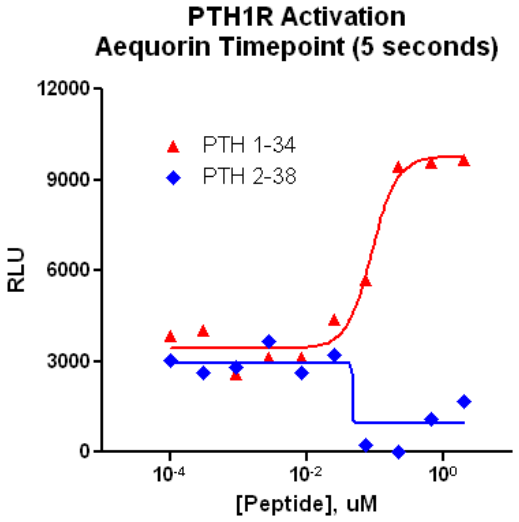
Stimulate cells in 384 format using 384-channel dispenser

Simultaneously gather “flash” and “Glo” luminescence in kinetic mode using FDSS µCell

Multiplexing of GloSensor™ cAMP Assay & Aequorin Reveals Selective Signaling to Gαs Using PTH (2-38) Ligand

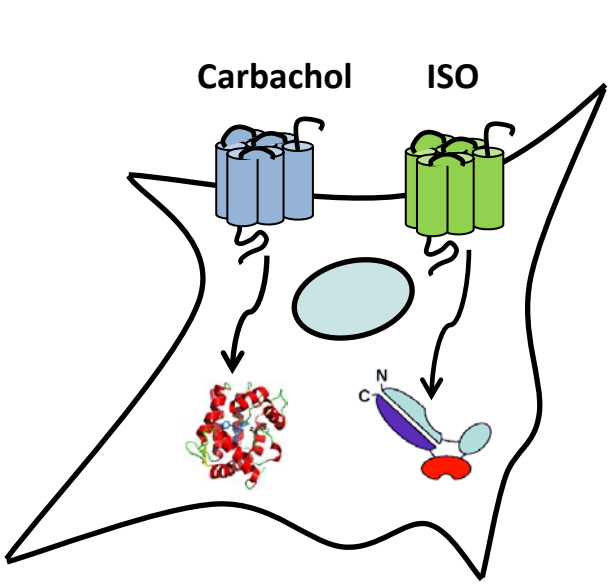


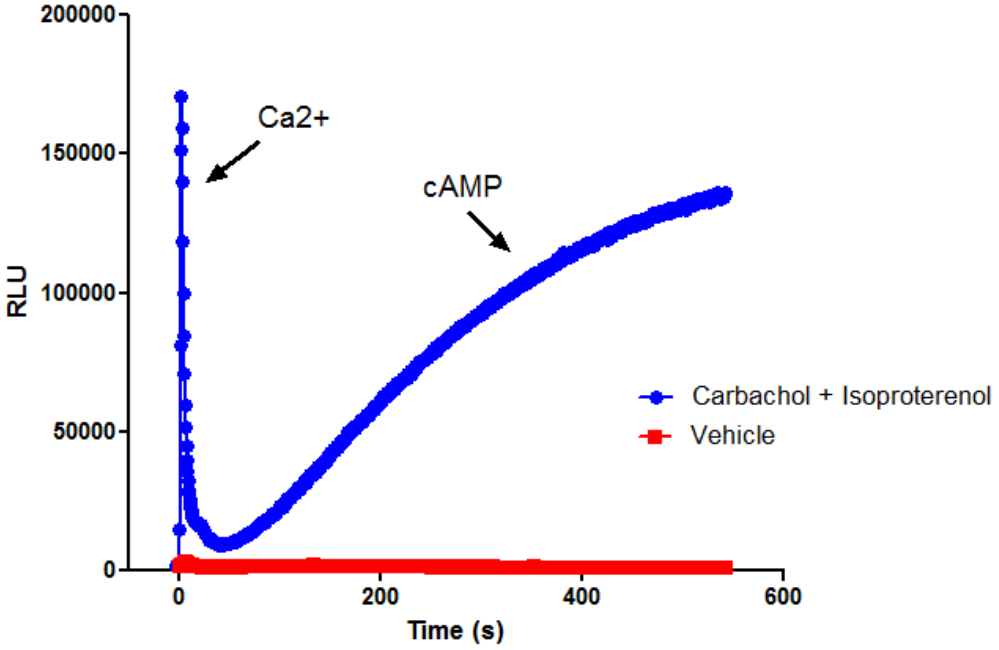




Multiplexed Analysis of Two Endogenous GPCRs in HEK293 Cells

GloSensor™ cAMP Assay + aequorin double stable cell line (HEK293)



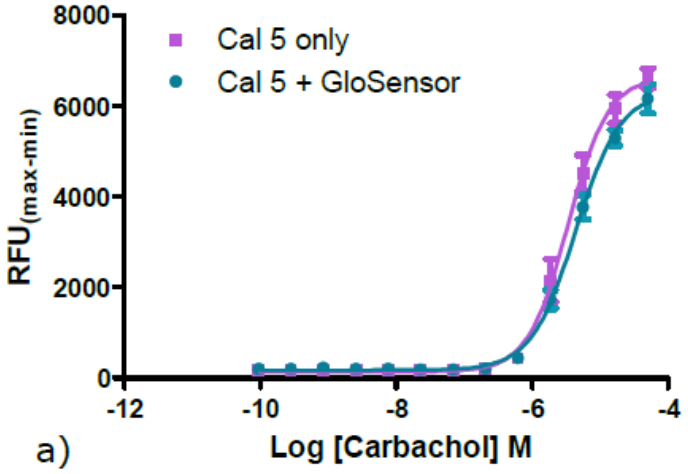


GloSensor™ cAMP Assay + Calcium Dye Multiplex

Promega

Multiplexing endogenous Gq- and Gs-coupled GPCR assays

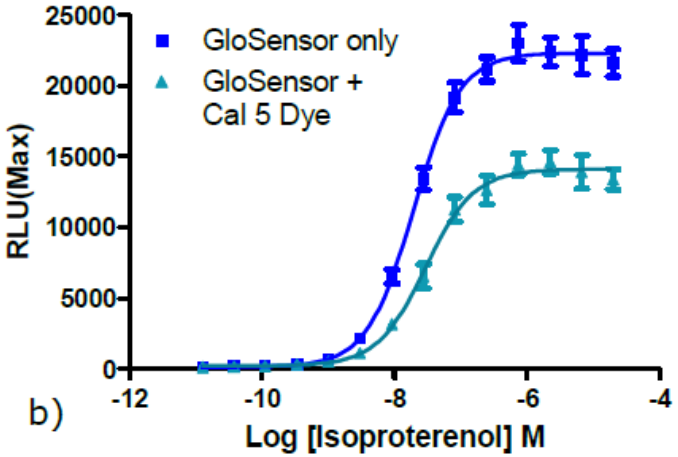
Calcium 5 Kit: Endogenous response
Muscarinic M3 receptor in 22F HEK 293 Cells



a)

EC_{50} Values	Dye Only	Dye + Glo
	3.374e-006	4.375e-006

GloSensor Assay: Endogenous response
β2 Adrenoceptor in 22F HEK 293 cells



b)

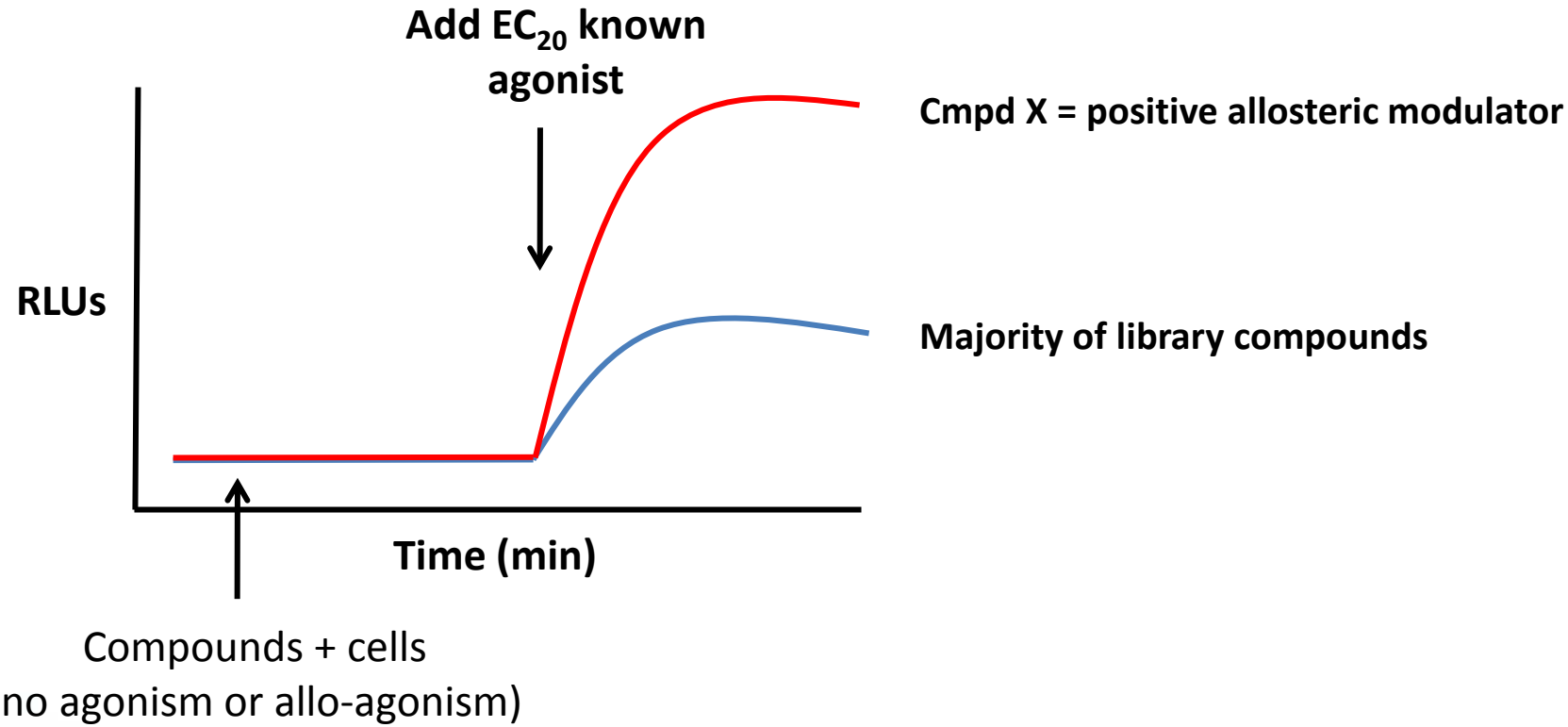
EC_{50} values	Glo Only	Glo + Dye
	1.946e-008	2.961e-008

Slide courtesy of Molecular Devices; exp. performed on FLIPR TETRA

Novel Screening Formats Using GloSensor™ cAMP Assay

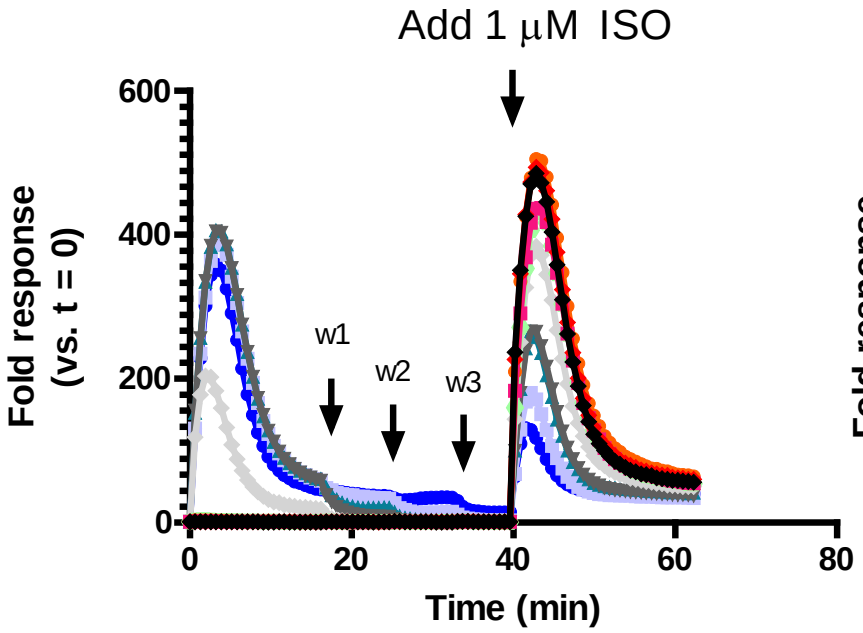
Promega

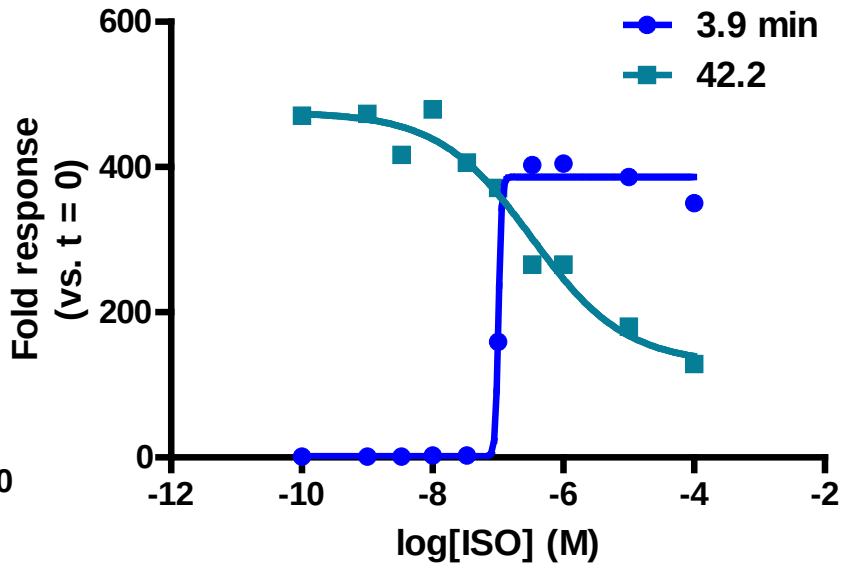
Screens for allosteric modulators of GPCR (1)



1. Pantel *et al.* (2011) *European Journal of Pharmacology* 660, p.139-147.

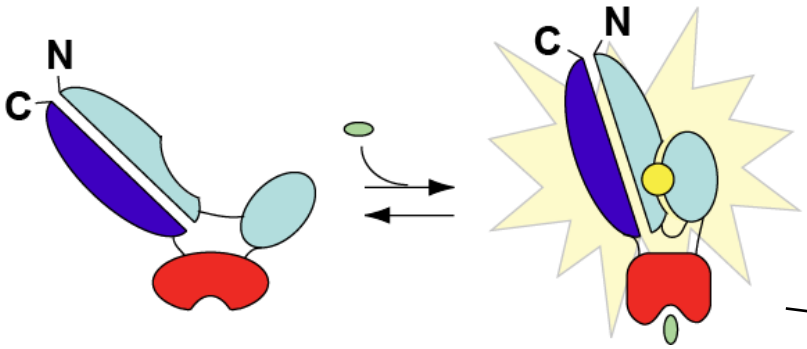
Monitoring Desensitization/Internalization



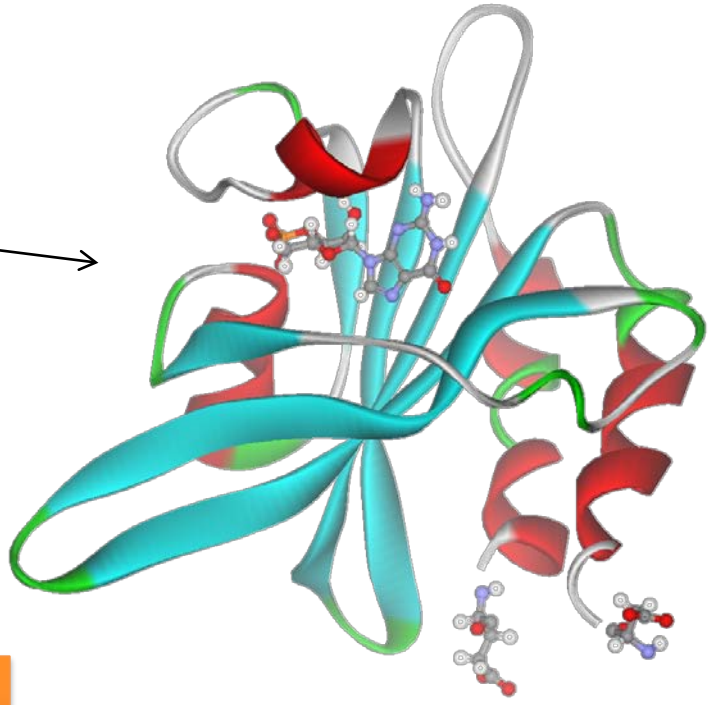


Transient expression in HEK293 cells; n = 1 per trace

GloSensor™ cGMP Assay*



Human PDE5A structure

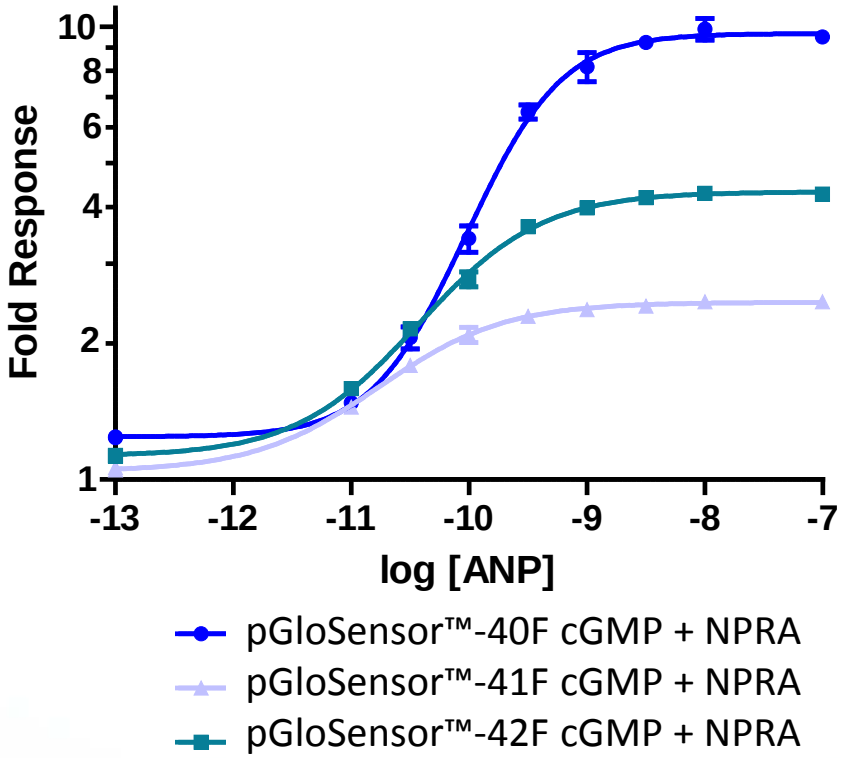


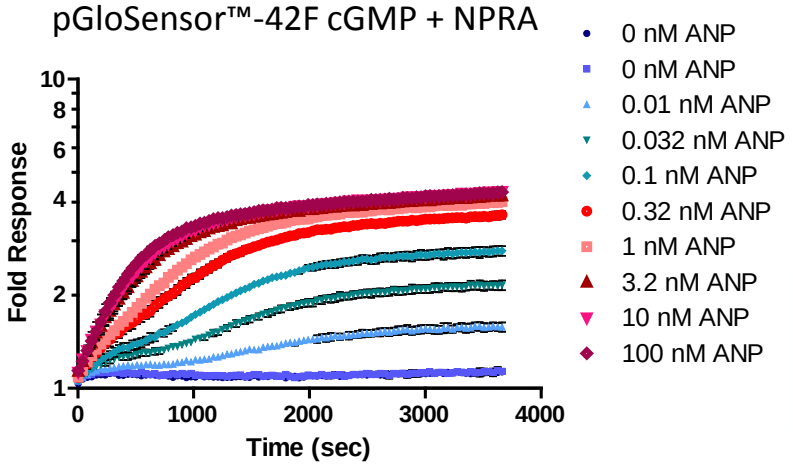
Analyte binding domains:
Human PDE5A (154-308)
Human PDE5A (154-312)
Human PRKG1 (85-328)

*pGloSensor™ cGMP Plasmids are available as custom research materials. Send inquiries to: CAS@promega.com

GloSensor™ cGMP Assay in HEK293 Cells

ANP dose-response curve with overexpressed NPRA



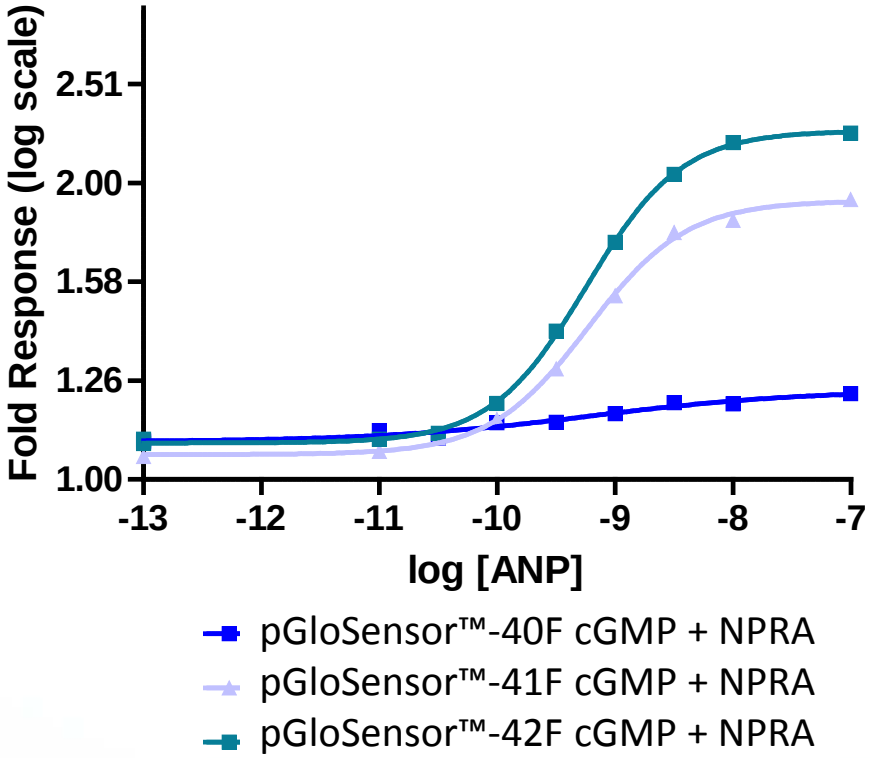


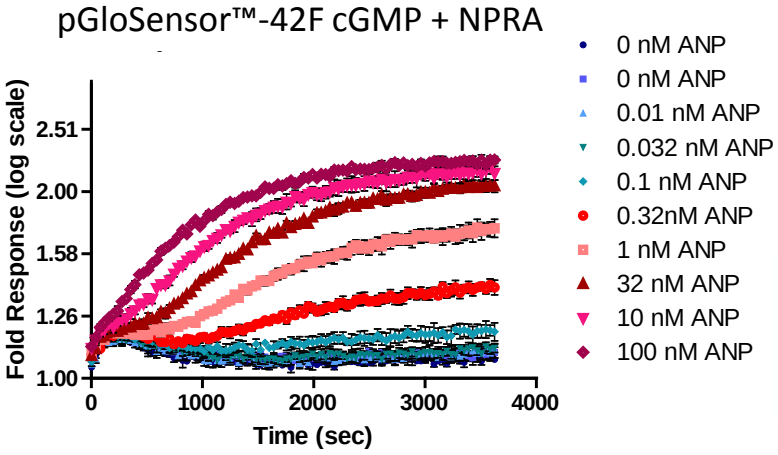
Log EC_{50}	EC_{50}
-9.66 ± 0.05	228pM
-10.09 ± 0.04	81.9pM
-10.54 ± 0.04	29.0pM

Transient expression in HEK293; values +/- S.E.M. for N=3

GloSensor™ cGMP Assay in HEK293 Cells (con't.)

ANP dose-response curve for endogenous ANP receptors





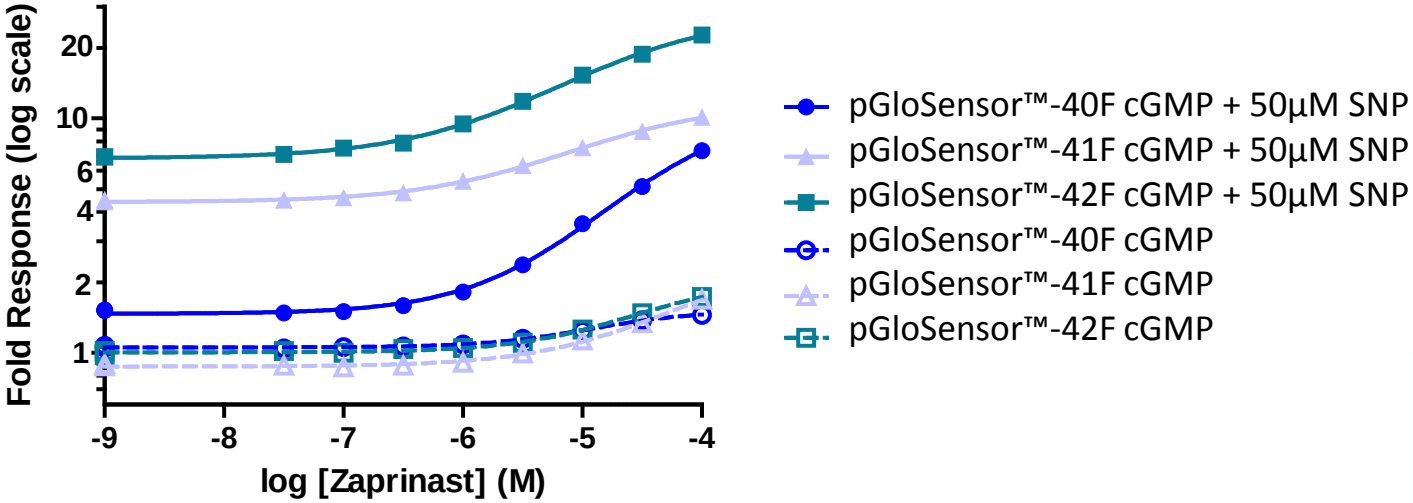
Log EC_{50}	EC_{50}
-9.14 ± 0.45	719pM
-9.099 ± 0.041	796pM
-9.107 ± 0.056	782pM

Transient expression in HEK293; values +/- S.E.M. for N=3

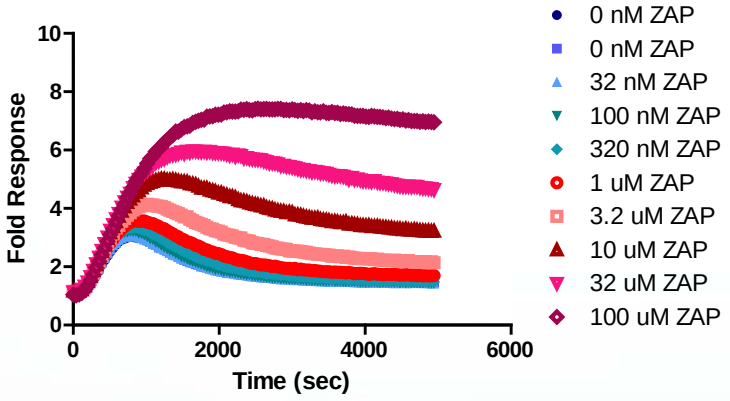
GloSensor™ cGMP Assay in HEK293 Cells (con't.)

Inhibition of endogenous PDEs by Zaprinast

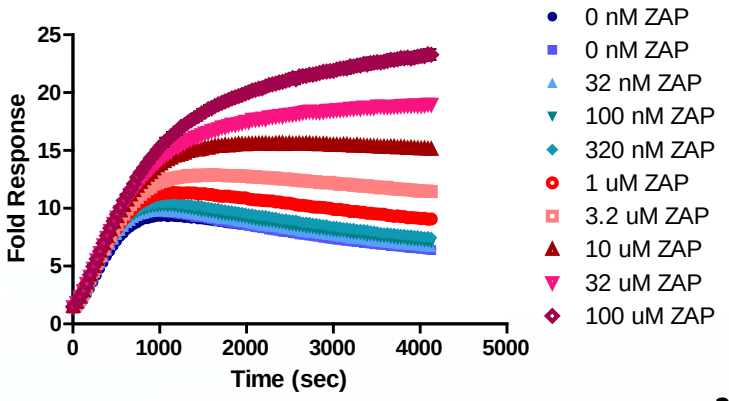
Transient expression in HEK293 cells; values +/- S.E.M. for n=3



pGloSensor™-40F cGMP + SNP/ZAP

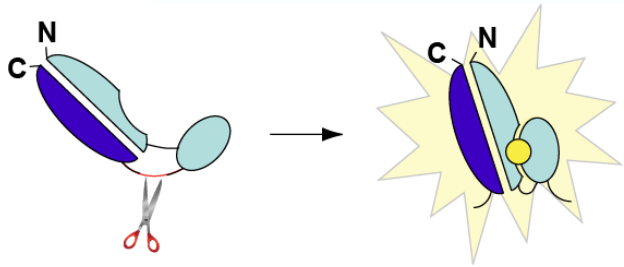


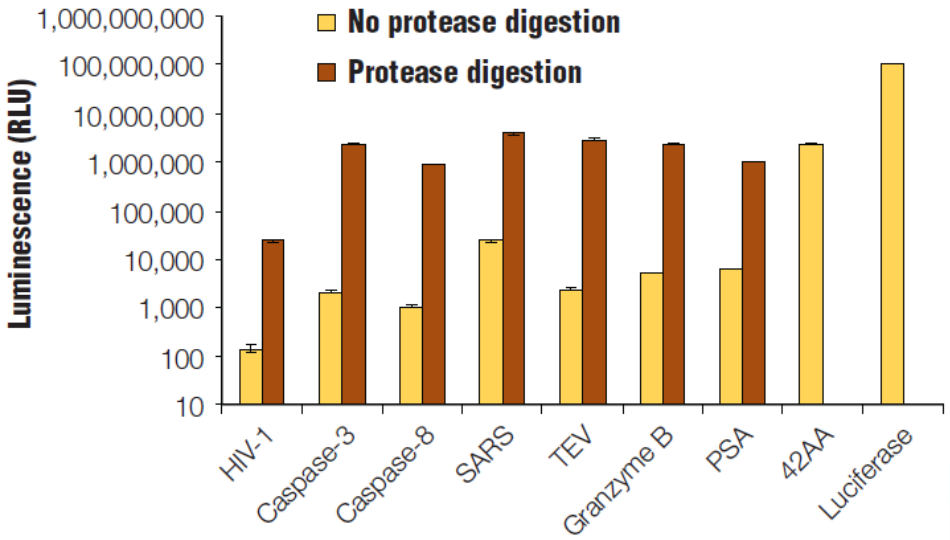
pGloSensor™-42F cGMP + SNP/ZAP



Protease-Glo™ Assay

We have also developed a protease sensor, based on the GloSensor™ platform, demonstrating effective in vitro activity for a host of protease recognitions sequences: **Protease-Glo™ Assay** (Cat.# G9451)



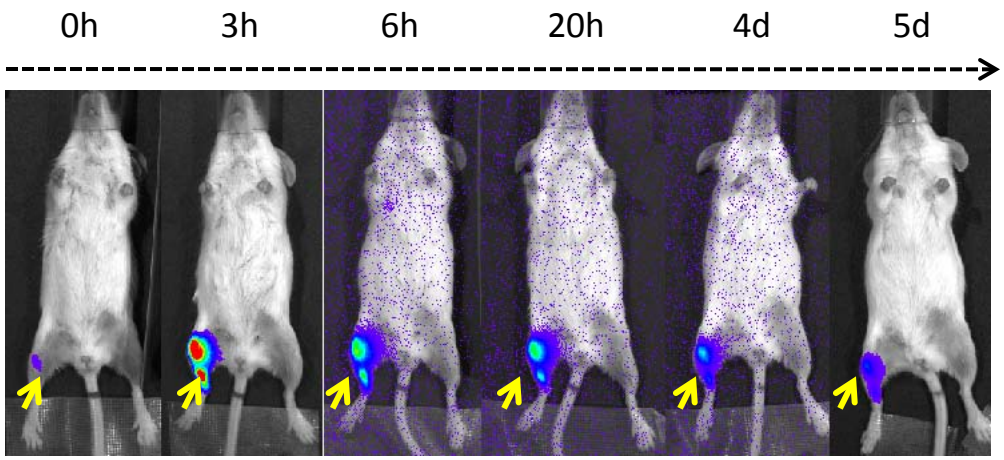


GGKSKL-DEVD↓G-MED	Caspase 3/7
GGKSKL-DDDDK↓-MED	Enterokinase
LE-LETD↓G-AW	Caspase 8
LE-ENLYFQ↓A-AW	TEV
LE-LEVLFQ↓GP-AW	Precission
LE-ANKASYQ↓SASTE-PW	PSA
LE-SITSAVLQ↓SGFRKMA-PW	SARS 3CL 1
LEMVPW	None

GloSensor™ Cell-Based Protease Application

Recently Dr Al Rehemtulla and colleagues at the University of Michigan demonstrated the use of the GloSensor™ technology to track apoptosis in live animals.

Time after TRAIL treatment



0 h	3 h	6 h	20 h	4 d	5 d
6.22E+06	7.39E+08	2.64E+08	2.02E+08	8.74E+07	4.08E+07

*pGloSensor™-30F DEVDG Plasmid is available as custom research material. Send inquiries to: CAS@promega.com

Cell-based biosensor Validation in Orthotopic Xenograft. Biosensor in breast bone metastases. Representative images taken at indicated time points of intratibial implanted 1833 reporter cells and photon counts per sec. Data presented at the Annual American Cancer Research meeting in Orlando (2011). Galbán, S *et al.* Imaging caspase dependent cell death as a surrogate for efficacy of cancer therapeutics, University of Michigan, Ann Arbor, MI Abstract #: LB-334

GloSensor™ Assays in Drug Discovery

Promega

Primary screening:

- Robust S/B & high Z´, validated in 384, 1536 & 3456-well formats
- Easily miniaturized to low volumes (1-10µl)
- Extremely cost effective in 96-to 3456-well format
- Novel screening formats (no requirement for cell lysis)
  - agonist screen → add EC_{80} of known agonist → antagonist screen
  - agonist screen → add EC_{20} of known agonist → allosteric modulator screen
- BacMam compatible
- Frozen cell compatible

Secondary screening & lead optimization:

- Quick & easy to perform
- Pharmacology is independent of assay format (96- to 1536-well)
- Kinetic profiling allows maximal information return on time investment
- Validated in primary and stem cells
- Demonstrated in in vivo imaging

Summary

GloSensor™ cAMP Assay

Live cell, non-lytic assay format with wide dynamic range and extreme sensitivity

- Simultaneous detection of full, partial and inverse agonists
- Gi-coupled receptor assays in the absence of added forskolin
- Sensitive detection of endogenous receptors (both Gs and Gi)
- Increased sensitivity, dynamic range & assay robustness (Z') vs. HTRF
- Novel screening formats
  - Allosteric modulator screens
  - cAMP & Ca^{2+} multiplex (Aequorin or Calcium 5 dye)
- Continuous measurement of cAMP synthesis and decay
  - Kinetic studies on receptor desensitization/cAMP turnover

GloSensor™ cGMP Assay

- Live cell, non-lytic assay format with extreme sensitivity
- No interference from cAMP or GTP
- Sensitive detection of GCs or PDEs in living cells

GloSensor™ Protease Assay

- General protease assay for use in vitro, in cell culture or in vivo

Key Scientific Publications

Promega

Allen, JA *et al.* Discovery of β-Arrestin-Biased Dopamine D2 Ligands for Probing Signal Transduction Pathways Essential for Antipsychotic Efficacy. *PNAS* **108** (45): 18488-18493, 2011 (DOI: 10.1073/pnas.1104807108)

Pantel, J *et al.* Development of a High Throughput Screen for Allosteric Modulators of 3 Melanocortin-4 Receptor Signaling Using a Real Time cAMP Assay. *Euro J Pharmacol* **660**: 139-147, 2011 (10.1016/j.ejphar.2011.01.031)

Binkowski, B *et al*. A Luminescent Biosensor with Increased Dynamic Range for Intracellular cAMP. *ACS Chem Biol* **6**: 1193–1197, 2011 (dx.doi.org/10.1021/cb200248h)

Buccioni, M, Marucci, G *et al.* Innovative functional cAMP assay for studying G protein-coupled receptors: application to the pharmacological characterization of GPR17. *Purinergic Signalling* **Publ online 20 July 2011** (DOI 10.1007/s11302-011-9245-8)

Rajagopal, S *et al.* Quantifying Ligand Bias at Seven-Transmembrane Receptors. *Mol Pharmacol* **80**(3):367-77, 2011 (DOI:10.1124/mol.111.072801)

Oka, T *et al.* Bioluminescence Technologies to Detect Calicivirus Protease Activity in Cell-free System and in Infected Cells. *Antiviral Research* **90**: 9–16, 2011 (DOI:10.1016/j.antiviral.2011.02.002)

Key Scientific Publications (con't.)

Nobles, KN *et al.* Distinct Phosphorylation Sites on the β2-Adrenergic Receptor Establish a Barcode That Encodes Differential Functions of β-Arrestin *Science Signaling* 4 (185), ra51, 2011 (DOI: 10.1126/scisignal.2001707)

Binkowski, B, Fan, F, and Wood, KV *Luminescent Biosensors for Real-Time Monitoring of Intracellular cAMP*, In: Signal Transduction Protocols, Series: Methods in Molecular Biology, Vol. 756, 263-271, Luttrell, LM and Ferguson, SSG, Eds, 2011 (ISBN 978-1-61779-159-8)

Hill, S, Williams, C, and May, Lauren Insights into GPCR pharmacology from the measurement of changes in intracellular cyclic AMP; advantages and pitfalls of differing methodologies. *Brit J Pharmacol* **161**(6): 1266-1275, 2010 (DOI: 10.1111/j.1476-5381.2010.00779.x)

Kimple, AJ *et al.* Structural Determinants of G-protein a Subunit Selectivity by Regulator of G-protein Signaling 2 (RGS2). *J Biol Chem* **284**: 19402-19411, 2009 (DOI: 10.1074/jbc.M109.024711)

Binkowski, B *et al*. Engineered Luciferases for Molecular Sensing in Living Cells. *Curr Opin Biotechnol* **20**(1): 14-18, 2009 (DOI:10.1016/j.copbio.2009.02.013)

Fan, F, Binkowski, B *et al.* Novel Genetically Encoded Biosensors Using Firefly Luciferase. *ACS Chem Biol* **3** (6): 346–351, 2008

Wigdal, S *et al.* A Novel Bioluminescent Protease Assay Using Engineered Firefly Luciferase. *Curr Chem Genomics* **1**: 94-106, 2008