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

New N- & C-termini
Fuse wt N- & C-termini

cAMP & cGMP Detection:
Analyte binding domain

Protease Detection:
Peptides

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

2. Mix compounds & cells

3. Kinetic or end-point measurements
GloSensor™ cAMP Assay

Analyte binding domain:
cAMP binding domain B
from human PKA regulatory
subunit type IIβ

Human RIIβB structure
GloSensor™ cAMP Variants In Vitro

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

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

<table>
<thead>
<tr>
<th></th>
<th>20F</th>
<th>22F</th>
</tr>
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<tbody>
<tr>
<td>EC₅₀ (µM)</td>
<td>0.3</td>
<td>9.0</td>
</tr>
<tr>
<td>Detection range</td>
<td>0.003-10µM</td>
<td>0.003-100µM</td>
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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

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Full, Partial & Inverse Agonist Detection

pGloSensor™-22F + ADRB2

Full, partial and inverse agonist detection in the same experiment

pGloSensor™-20F + ADRB2

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**

**pGloSensor™-22F cAMP construct**

**pGloSensor™-20F cAMP construct**
Measuring Two Second Messenger Pathways in Real-time

PTH (1-34)

\[ \begin{align*}
& \text{cAMP} \\
\rightarrow & \quad \text{Ca}^{2+} \\
\rightarrow & \quad \text{GloSensor™} \\
\rightarrow & \quad \text{Aequorin}
\end{align*} \]

PTH (2-38)

\[ \begin{align*}
& \text{cAMP} \\
\rightarrow & \quad \text{Ca}^{2+} \\
\rightarrow & \quad \text{GloSensor™} \\
\rightarrow & \quad \text{Aequorin}
\end{align*} \]

Ca2+ cAMP

Luminescence

Seconds Minutes
**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

Multiplexing endogenous Gq- and Gs-coupled GPCR assays

Slide courtesy of Molecular Devices; exp. performed on FLIPR TETRA
Novel Screening Formats Using GloSensor™ cAMP Assay

Screens for allosteric modulators of GPCR (1)

Compounds + cells
(no agonism or allo-agonism)

Add EC$_{20}$ known agonist

Cmpd X = positive allosteric modulator

Majority of library compounds

Monitoring Desensitization/Internalization

Transient expression in HEK293 cells; n = 1 per trace

Add 1 μM ISO

Fold response (vs. t = 0)

Time (min)

Fold response (vs. t = 0)

log[ISO] (M)

3.9 min

42.2

w1

w2

w3

3.9 min

42.2
GloSensor™ cGMP Assay*

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

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

Log EC<sub>50</sub> | EC<sub>50</sub>
--- | ---
-9.66 ± 0.05 | 228pM
-10.09 ± 0.04 | 81.9pM
-10.54 ± 0.04 | 29.0pM
GloSensor™ cGMP Assay in HEK293 Cells (con’t.)

ANP dose-response curve for **endogenous** ANP receptors

![ANP dose-response curve](image)

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

Cell-based biosensor Validation in Orthotopic Xenograft.

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

**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\textsubscript{80} of known agonist → antagonist screen
  - agonist screen → add EC\textsubscript{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


Key Scientific Publications (con’t.)


