# *Custom Assay Services from Promega*

# Outline

Promega & Custom Assay Services Team

What We Do

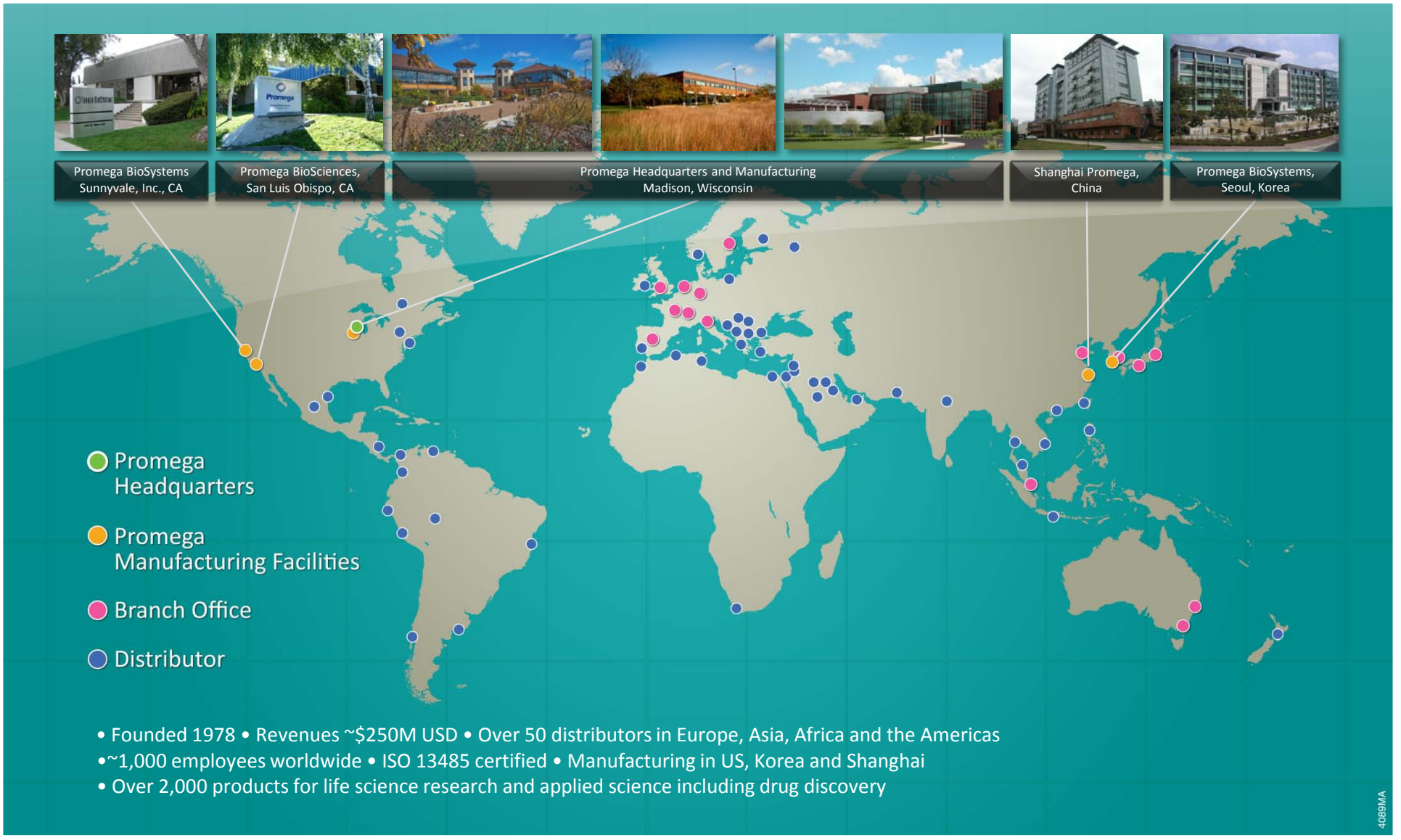
How We Do It

Case Studies

Support

Future Directions

# World-Wide Connection



# Solid Infrastructure and Intellectual Foundation


**Quality System Overview**

Promega Madison achieved ISO 9001 Certification in 1998, ISO 13485 in 2006

Quality system approach – complements and supports business objectives

Process driven with heavy emphasis on measures to drive performance

**Intellectual Property**

- 430 Cellular Analysis Patents
- 244 Genomics Patents
- 153 Genetic Identity, Other Patents
- 51 Proteomics Patents

# World-Class Capabilities in Custom Assay Development

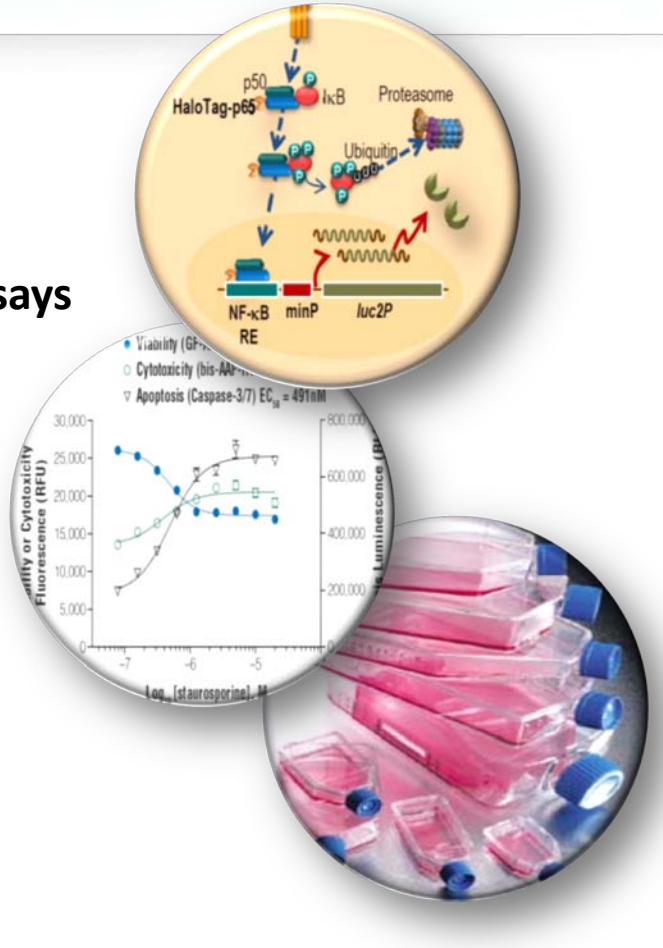
**Promega has the tools, expert staff and state-of-the-art facilities to support complete custom solutions for biochemical & cell-based assays**

**CELL ENGINEERING**
Target expression in both normal & disease states

**ASSAY DEVELOPMENT & QUALIFICATION**
Target class expertise & multiple assay formats

**ASSAY READY CELLS IN-SCALE**
Pharmacological validation & unparalleled client support

And we provide all the post-delivery support to ensure your assay works in your hands



# Value Proposition to Drug Developers

**Let us partner with you to develop a complete biology-driven, Promega technology-enabled custom solution.**

Faster time to data

Fewer in-house resources consumed & increased productivity

Flexible approach & offerings –
we can adapt our process to meet your needs

# Flexible by Design

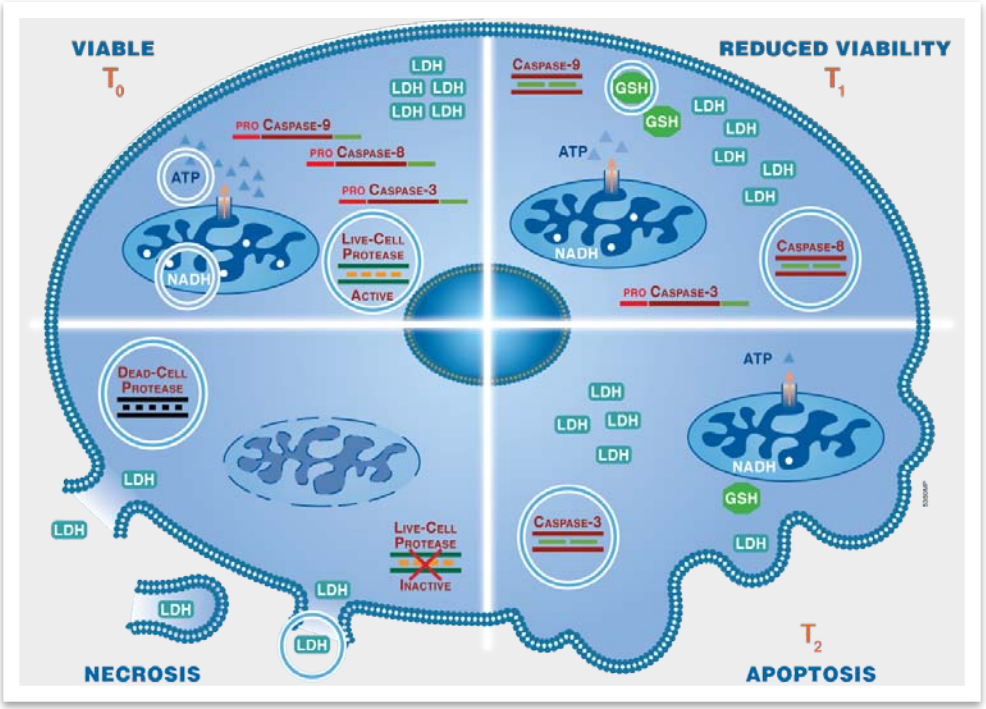
**Whether your need is in small molecule drug discovery or developing biologics, we can create an assay that suits your requirements:**

Cell-based assays

Biochemical assays

Multiple detection technologies

Multiplexed readouts for increased biological understanding



# Forward Thinking…

**We have solutions for your needs today and innovative thinking for your needs tomorrow.**

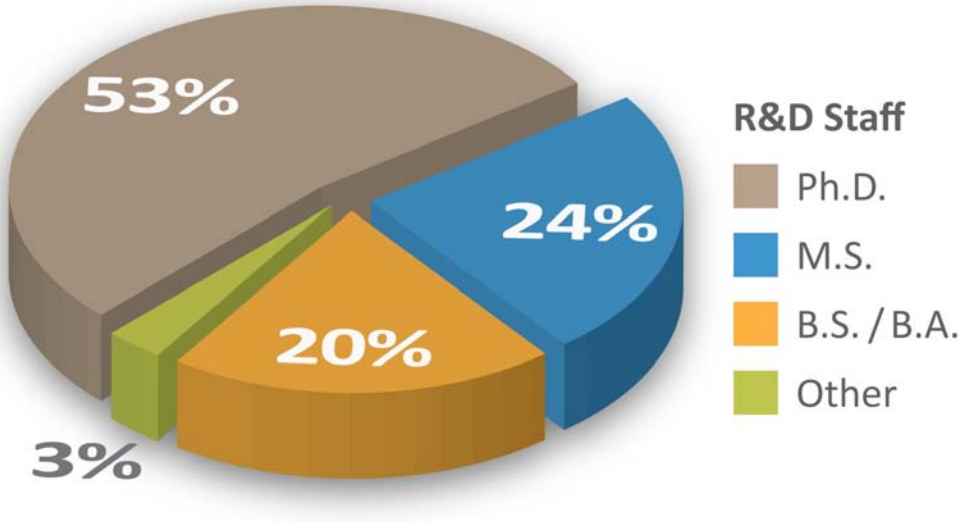
Our scientists and business teams continue to enter into research collaborations with leading laboratories world-wide to investigate novel solutions, such as stem cells & whole animal imaging

# Scientific & Assay Development Expertise

**Global Leader in Bioluminescence Chemistry**

**R&D facilities located in:**
Madison, WI
San Luis Obispo, CA
Seoul, Korea
Sunnyvale, CA

**Extensive experience in developing assays for:**
Genetic reporters
Cell viability & apoptosis
ADME/Tox
GPCRs
Kinases
In vivo imaging
Detection instrumentation



# Transparent Collaboration Process

## The Client is the Partner throughout all steps

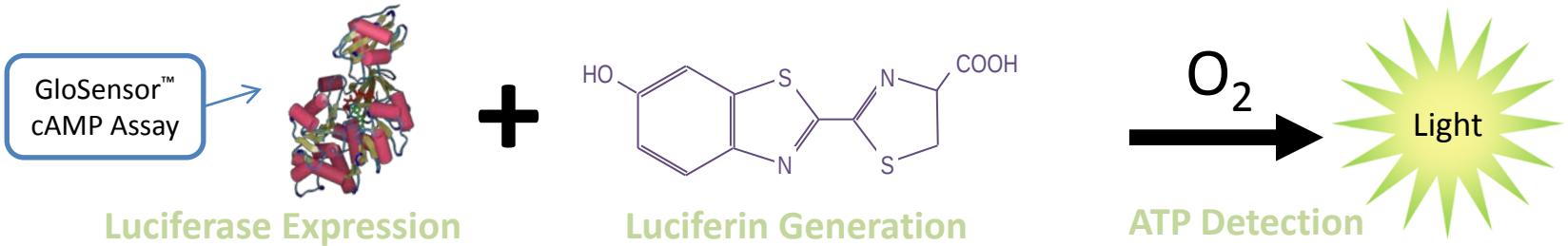
# Key Technologies

## Comprehensive Portfolio of Assays for Cell Analysis

- CellTiter-Glo® Assay for monitoring cell viability
- Caspase-Glo® Assay for monitoring apoptosis
- Multiplexed viability, toxicity and apoptosis assays for more comprehensive monitoring of cellular health
- Luciferase reporter assays for cellular pathway analysis
- HaloTag® portfolio of live-cell protein trafficking, protein labeling, and purification tools
- GloSensor™ technology for the live-cell monitoring of cAMP, an important second messenger in GPCR signaling

*And a comprehensive portfolio of reagents for genomics, proteomics and applied markets*

# Applying the Power of Bioluminescence to Biology




### Cell-Based Assays

ONE-Glo™ Luciferase Assay System
Bright-Glo™ Luciferase Assay System
Steady-Glo® Luciferase Assay System
Dual-Glo™ Luciferase Assay System

### Fluorescent Multiplex-Friendly Cell Health Assays

CellTiter-Fluor™ Cell Viability Assay
CytoTox-Fluor™ Cytotoxicity Assay
MultiTox-Fluor™ Multiplex Cytotoxicity Assay

### Cell-Based Assays

ApoTox-Glo™ Triplex Assay
ApoLive-Glo™ Multiplex Assay
CytoTox-Glo™ Cytotoxicity Assay
Caspase-Glo® 3/7 Assay System
Caspase-Glo 8 & 9 Assay System
GSH-Glo™ Glutathione Assay
MultiTox-Glo Multiple Cytotoxicity Assay
P450-Glo™ Assay Screening System
Proteasome-Glo™ Cell Based Assays

### Biochemical Assays

Calpain-Glo™ Assay
Caspase-Glo 2 & 6 Assay
DPPIV-Glo™ Assay
DUB-Glo™
MAO-Glo™ Assay
P450-Glo™ Assay Screening Systems
Proteasome-Glo™ Assays
UGT-Glo™ Assay

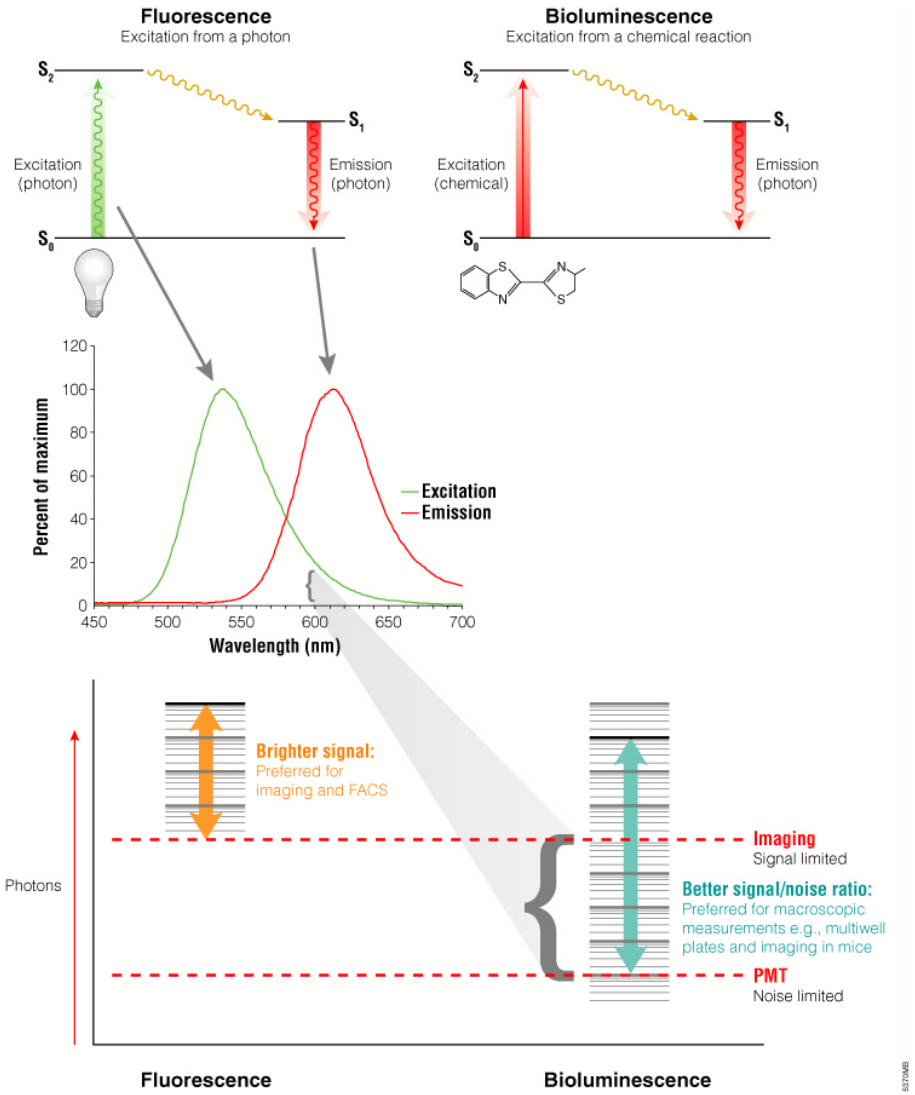
### Cell-Based Assays

CellTiter-Glo® Luminescent Cell Viability
BacTiter-Glo® Microbial Cell Viability Assay
cAMP-Glo™ Assay

### Biochemical Assays

ADP-Glo™ Kinase Assay
Kinase-Glo® Assay
Kinase-Glo® Plus Assay
Kinase-Glo® Max Kinase Assay
PDE-Glo™ Assay
Pgp-Glo™ Assay System

# The Physics of Bioluminescence is Key to Better Biology




**Luminescent assays avoid issues of fluorescence interference and offer a greater dynamic range when needed**

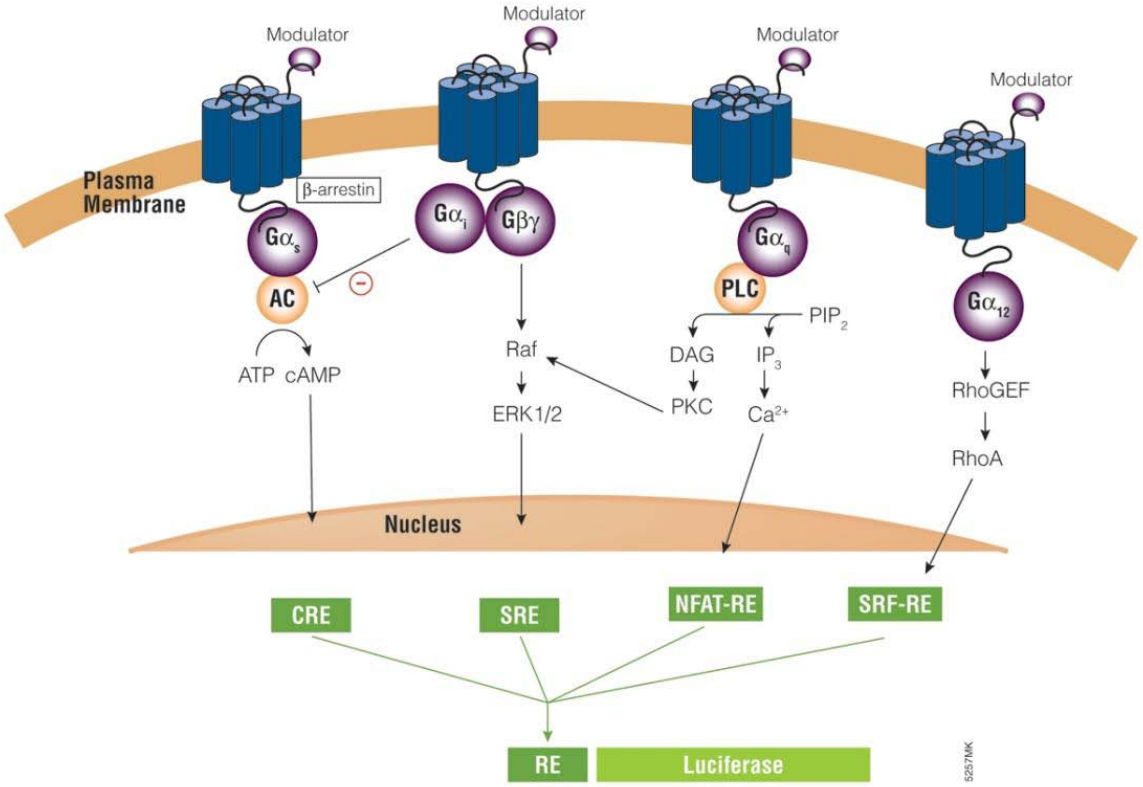
Better signal-to-noise ratio for multi-well plate assays & in vivo imaging in mice

Ultra-Glo is the best luciferase for cell-based luminescence protocols

Easier multiplexing allowed when luminescence & fluorescence combined

Luminescence detection transfers from biochemical to cell-based to animal studies seamlessly

# Solutions for GPCR Biology




**GloSensor™ cAMP Assay:**
Live-cell HTS assay

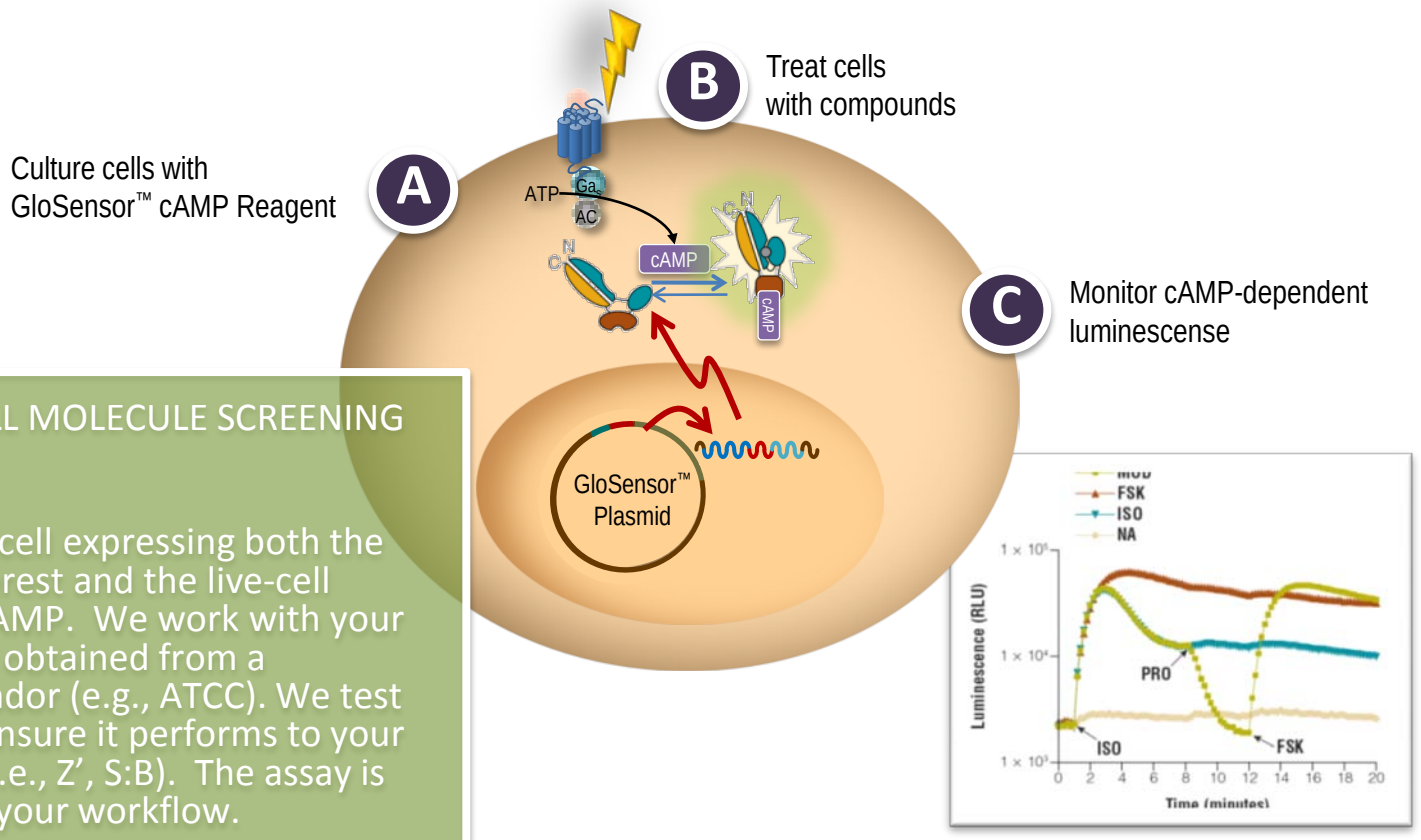
**cAMP-Glo™ Assay:**
Lytic HTS assay

**Luciferase reporter assays for:**
G12/13
cAMP-RE
$Ca^{2+}$-RE
ERK: SRE-RE

**HaloTag® Assay:**
Receptor internalization via protein trafficking

# Monitor cAMP Levels in Real-time with a Live-cell, Non-lytic Bioluminescent Assay

## Monitor Gs- and Gi-coupled GPCRs Using GloSensor™ cAMP Assay




EXAMPLE SMALL MOLECULE SCREENING SOLUTION:

Create a stable cell expressing both the receptor of interest and the live-cell biosensor for cAMP. We work with your cell line or cells obtained from a commercial vendor (e.g., ATCC). We test the assay and ensure it performs to your specifications (i.e., Z', S:B). The assay is ready to run in your workflow.

# GloSensor™ cAMP Assay is as Sensitive as an Immunoassay

**Day 1:** Plate HEK293 15,000 cells/well, 96-well plate

↓

**Day 2:** Transient transfection using Mirus TransIT-LT1

↓

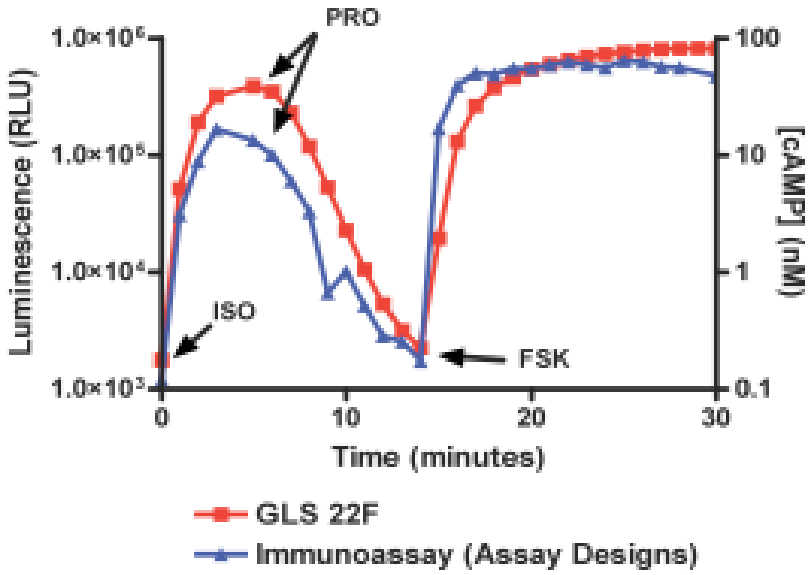
**Day 3**: GloSensor cAMP, equilibrate w/ substrate for 2 hrs at RT; EIA, incubate cells for 2 hrs in medium alone

↓

GloSensor cAMP, sequential addition of compounds & continuous RLU measurement; EIA, same, except addition of 0.1 M HCl to separate samples at indicated time pts; 28°C

↓

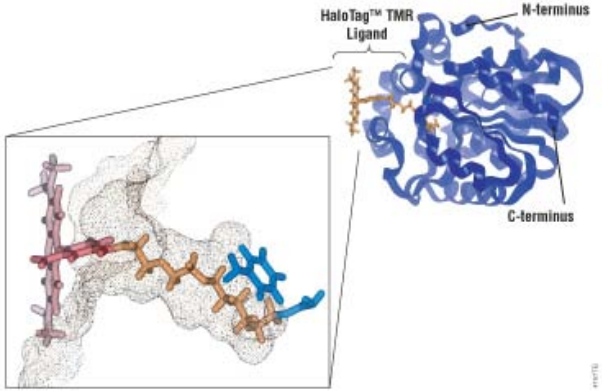
EIA dilutions: 2-fold dilutions w/ buffer 1-6’; 4-fold dilutions w/ buffer 15-30’

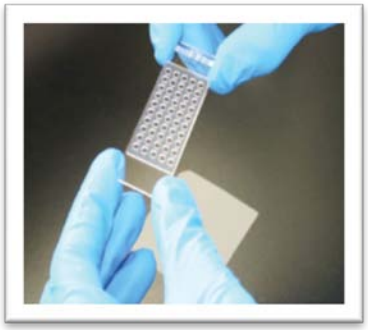


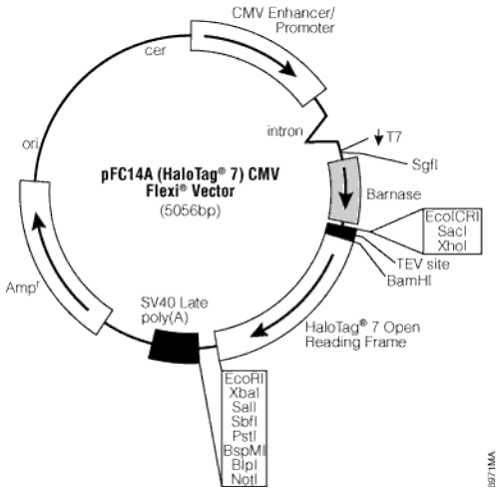

Assay Designs, Cat.# 901-066, acetylated

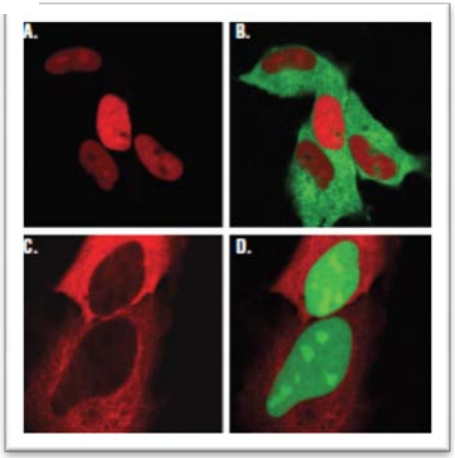
# Monitor Protein Trafficking, Label Live Cells and So Much More

## Using HaloTag® Protein Labeling Technology






**A wide variety of Flexi Vectors, Ligands and other label tools allow:**

Live cell imaging

Protein localization

Protein trafficking

Protein capture

Cell-to-gel analysis

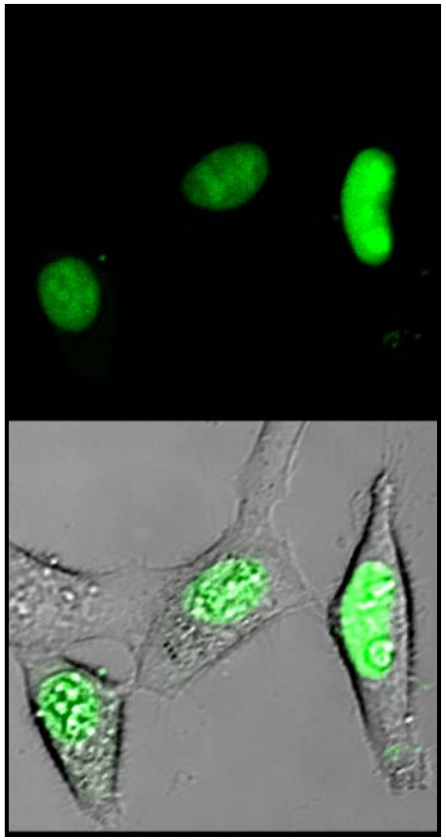
Protein:protein interaction analysis

Protein:DNA interaction analysis

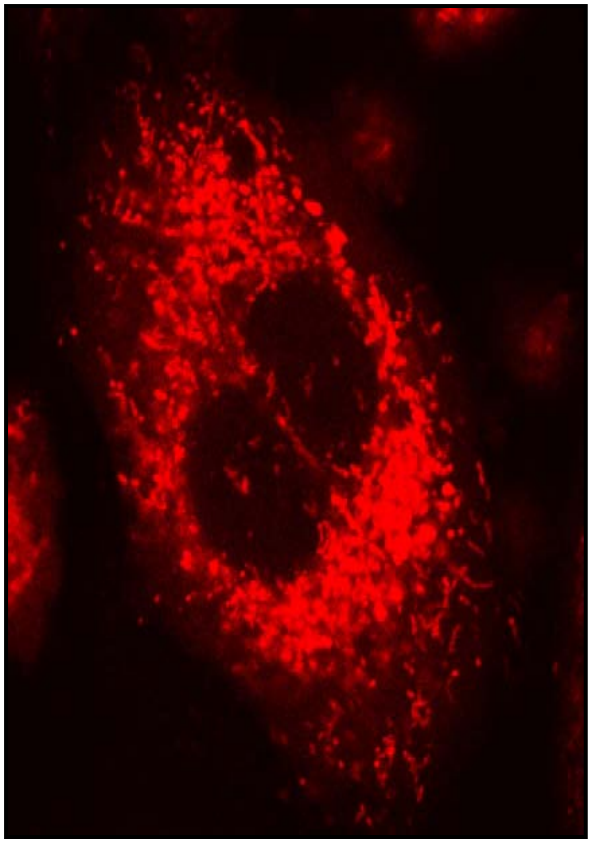
Protein purification

# Sub-cellular targeting of HaloTag fusions

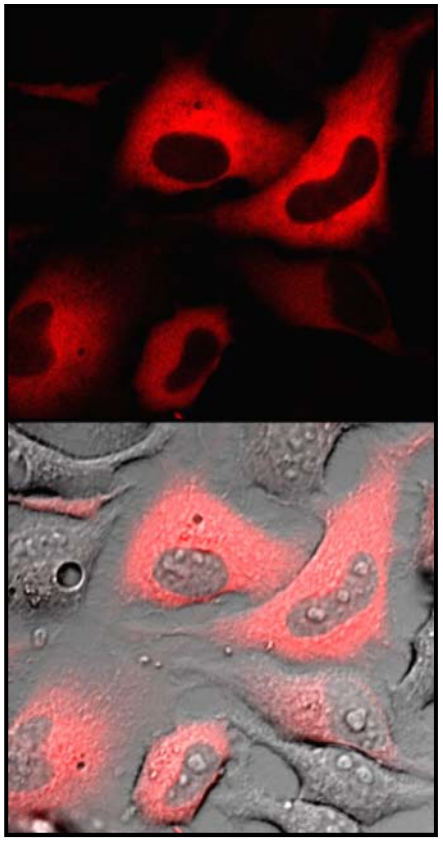
Nucleus
HaloTag - $NLS_3$



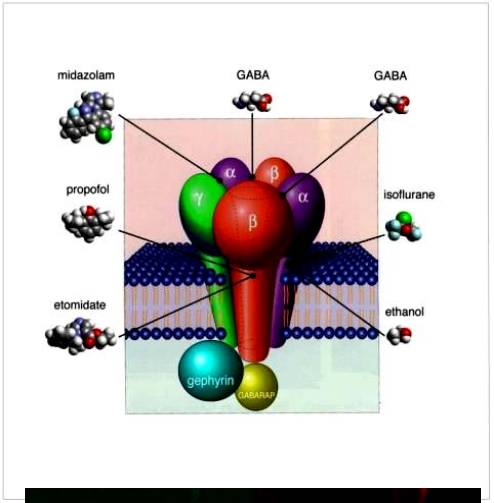
Mitochondria
Mito - HaloTag

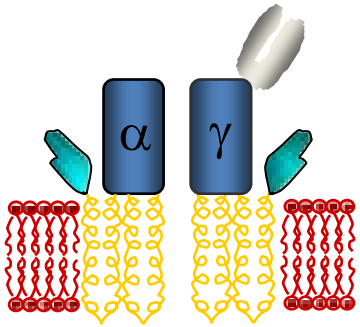


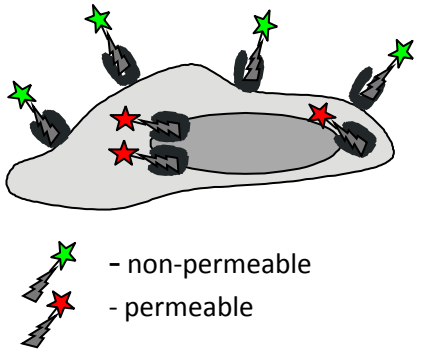
Cytosol
p65 - HaloTag

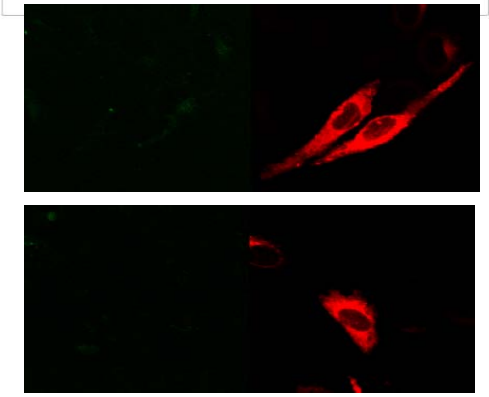


# $GABA_A$ receptor, a ligand-gated ion channel super family

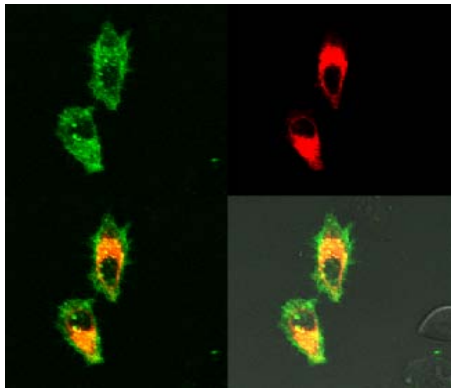


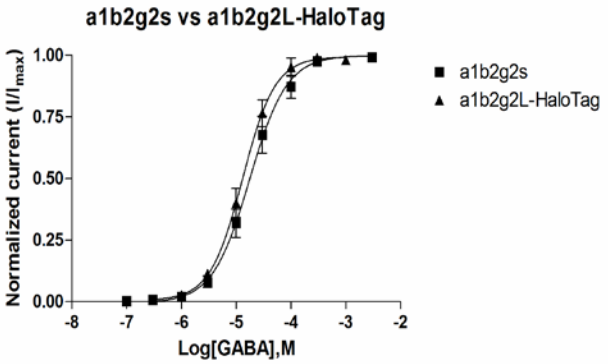



γ2L-HaloTag co-expressed with α1 or b2 does not traffic to cell surface

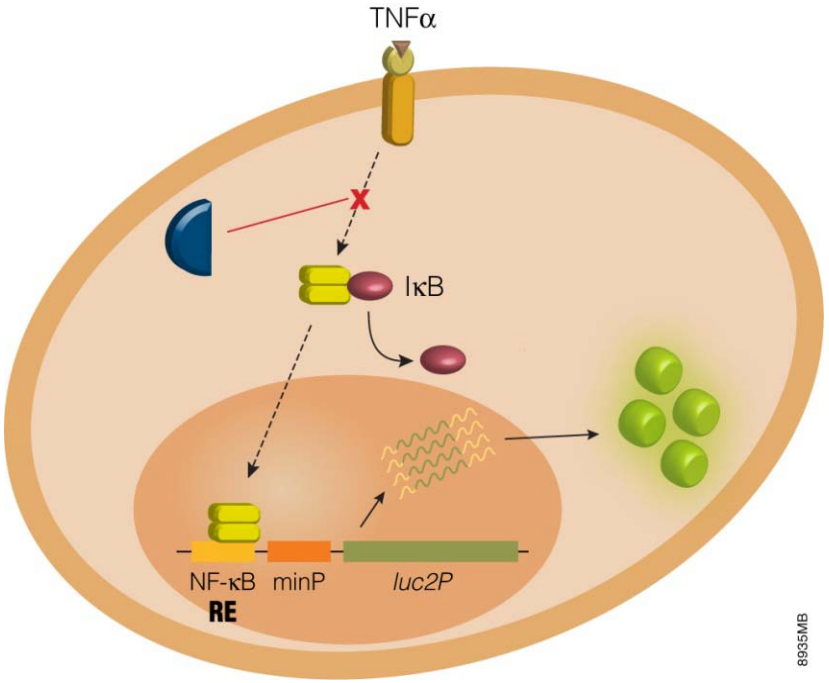


γ2L-HaloTag co-expressed with both α1 and β2 traffics to the cell surface

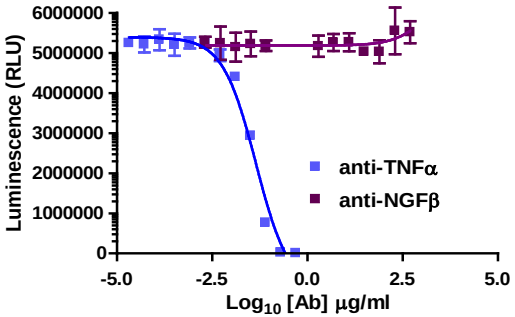



GABAAR pharmacology is not affected

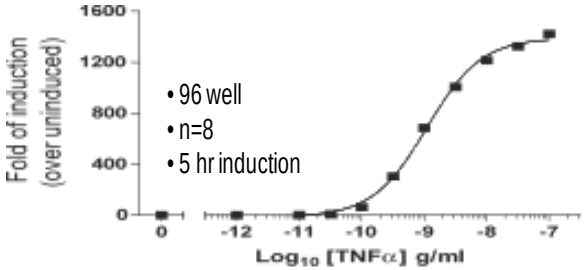
# NF-κB-Response Element Reporter Gene Assay

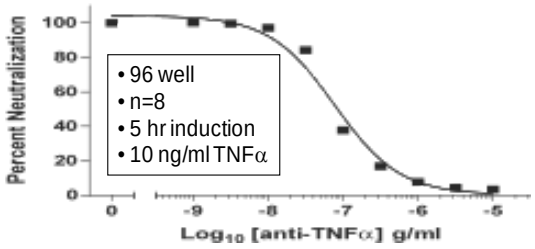



Specificity




TNFα/anti-TNFα bioactivity

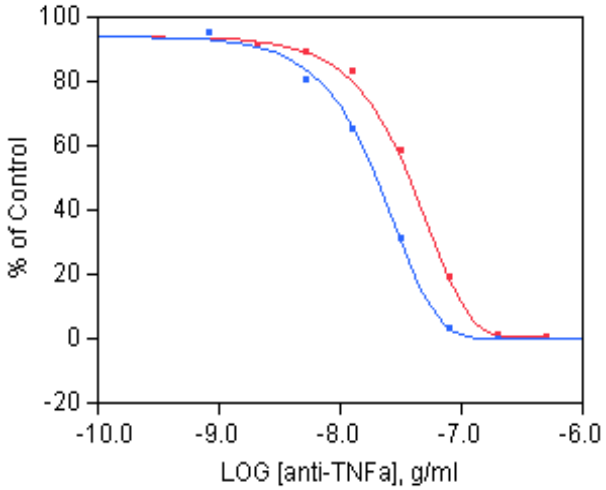


# Parallelism & Linearity Across Potencies

## Anti-TNFα Bioassay Using NF-κB-RE Luciferase Assay

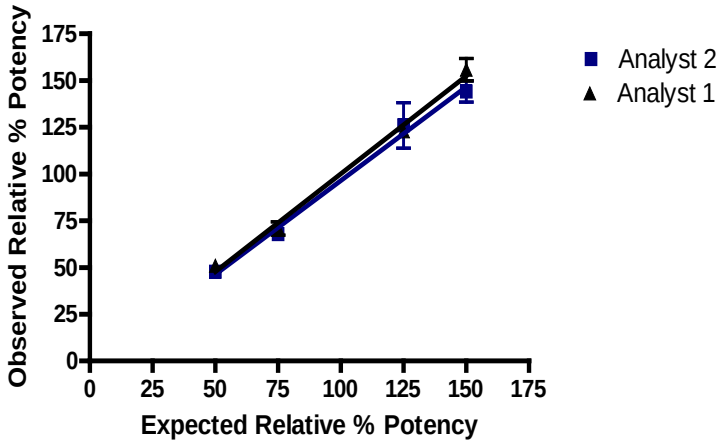
Example: Parallelism is observed for a 50% potency sample relative to a 100% reference sample




**Relative Potency**

| Parameter | Estimate |
|---|---|
| Log Standard EC50 | -7.6939 |
| Log Unknown EC50 | -7.4249 |
| Relative Potency | 0.53823 |
| Parallel Check (p >F) | 0.91758 |

Linearity is evident for the potency range




Parallelism analysis performed using JMP software (SAS Institute)

# Accuracy of Bioluminescent Anti-TNFα Bioassay

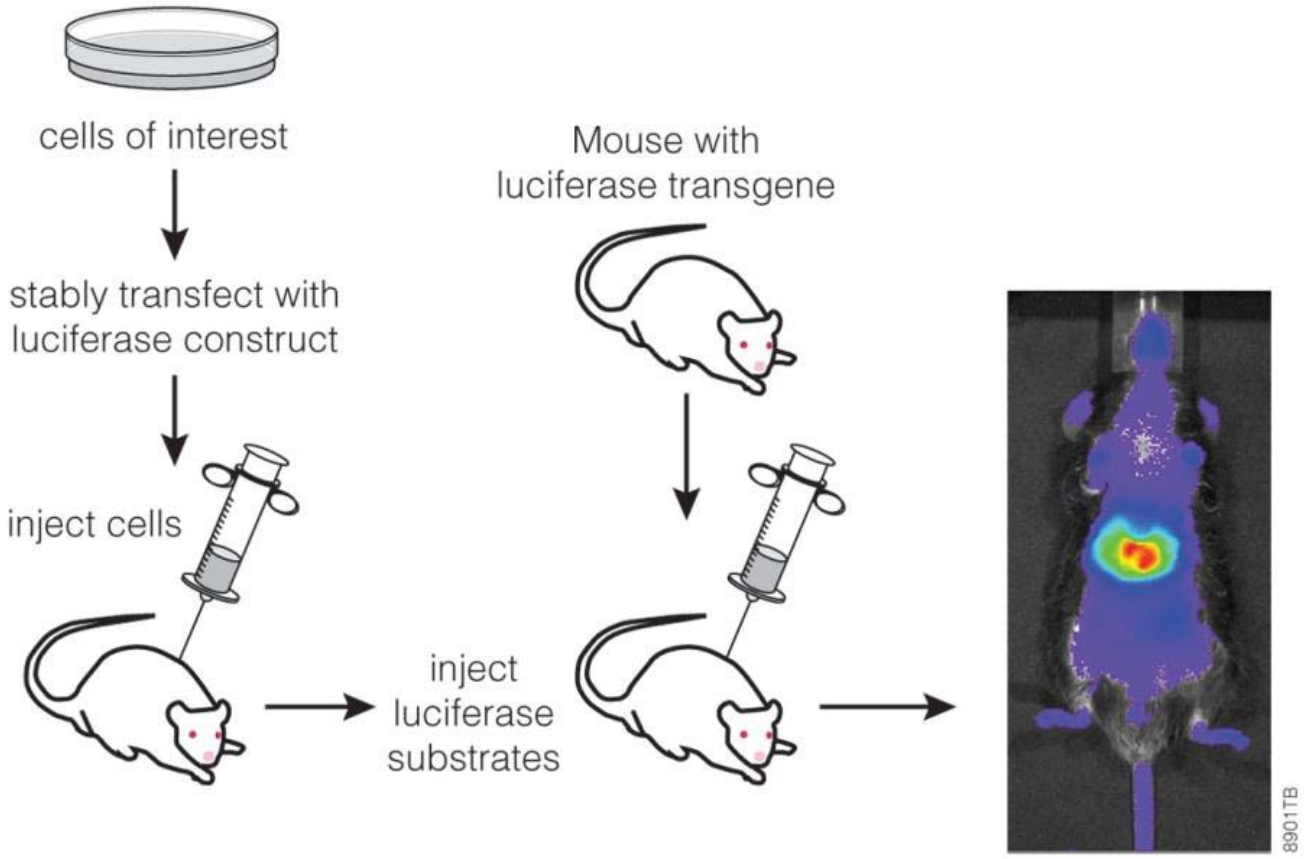
**NF-κB-RE reporter gene assay**

| Potency sample | Recovery Analyst 1 | Recovery Analyst 2 |
|---|---|---|
| 50% | 101.8% | 95.4% |
| 75% | 94.6% | 90.5% |
| 125% | 98.2% | 100.8% |
| 150% | 103.9% | 96.1% |

Data are means from 3 separate assays performed on 3 different days

# From Cells to In Vivo Imaging

**Luciferase technology is ideal for carrying assays into live animals**



# CASE STUDIES:
# Solutions for Small Molecule & Biologics Drug Discovery

# CASE STUDY: Custom Stable Cell Line Need for Small Molecule Drug Discovery Program

**A large pharmaceutical company was having difficulty developing a stable cell line in a specific cellular background due to technical issues.**

***Problem:*** Difficulty developing a stable cell; needed a cell line with sensitive readout for specific cell signal response

***Proposal:*** Create and validate HaloTag-containing stable cell line

Solution: Validated stable cell line in 12 weeks

***Result:*** Fewer resources expended by Client; successful assay when previous efforts failed; Client spent less in saved resources and effort in not troubleshooting assay development.

# CASE STUDY: Custom Bioassay Need for Drug Discovery in Biologics

**A global pharmaceutical company needed a more sensitive and easier-to-perform bioassay for pre-clinical and clinical phases of new drug development.**

***Problem:*** Laborious, poorly sensitive assay to support potential blockbuster drug in clinical trials and post-launch

***Proposal:*** Create a qualified luciferase reporter assay including training & transfer of assay to Client

***Solution:*** Qualified potency bioassay developed in 21 weeks

***Result:*** More sensitive & easier to perform lot release assay; Client not experienced in developing cell-based assays and concentrated on other aspects of the project while Promega developed the assay solution.

# Strategic Collaborations Team

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*The Strategic Collaborations Team is supported by a network of professionals within Promega. From the experienced R&D staff, to Custom Order Specialists, to business and legal management, we're committed to meeting your needs and exceeding your expectations.*

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# Worldwide Support

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## Manufacturing:

**Promega BioSciences, Inc.**
San Luis Obispo, CA, USA

**Promega BioSystems, Inc.**
Seoul, South Korea

**Promega BioSystems Sunnyvale, Inc.**
Sunnyvale, CA, USA

**Shanghai Promega Biological Products, Ltd.**
Shanghai, China

**Terso Solutions, Inc.**
Madison, WI, USA

## Branch Offices:

**Promega Corporation**
Sydney, Australia

**Promega (Beijing) Biotech Co., Ltd**
Beijing, China

**Promega France**
Lyon, France

**Promega GmbH**
Mannheim, Germany
(Austria)

**Promega Italia, S.r.l.**
Milan, Italy

**Promega KK**
Tokyo, Japan

**Promega Korea, Ltd**
Seoul, South Korea

**Promega Benelux b.v.**
Leiden, The Netherlands
(Belgium/Luxembourg)

**Promega Pte Ltd.**
Pacific Asia Region, Singapore

**Promega Biotech Ibérica, SL**
Madrid, Spain

**Promega Biotech AB**
Stockholm, Sweden
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