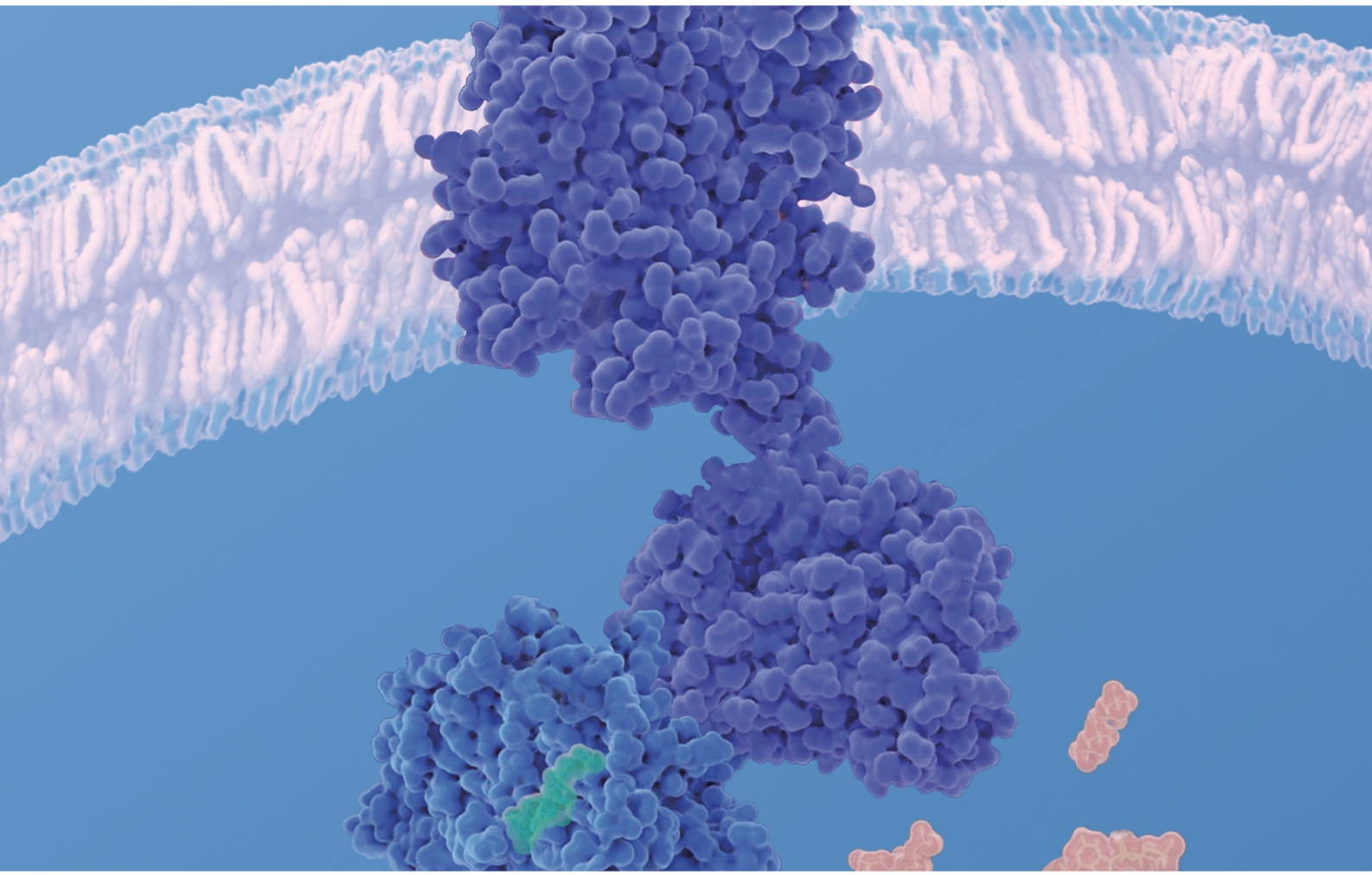
Advancing GPCR Drug Discovery

Flexible Solutions for Next-Generation Therapeutics



Innovative CRO+Explorer
ICE Bioscience

Advances in GPCR Targeting: A New Era in Drug Development

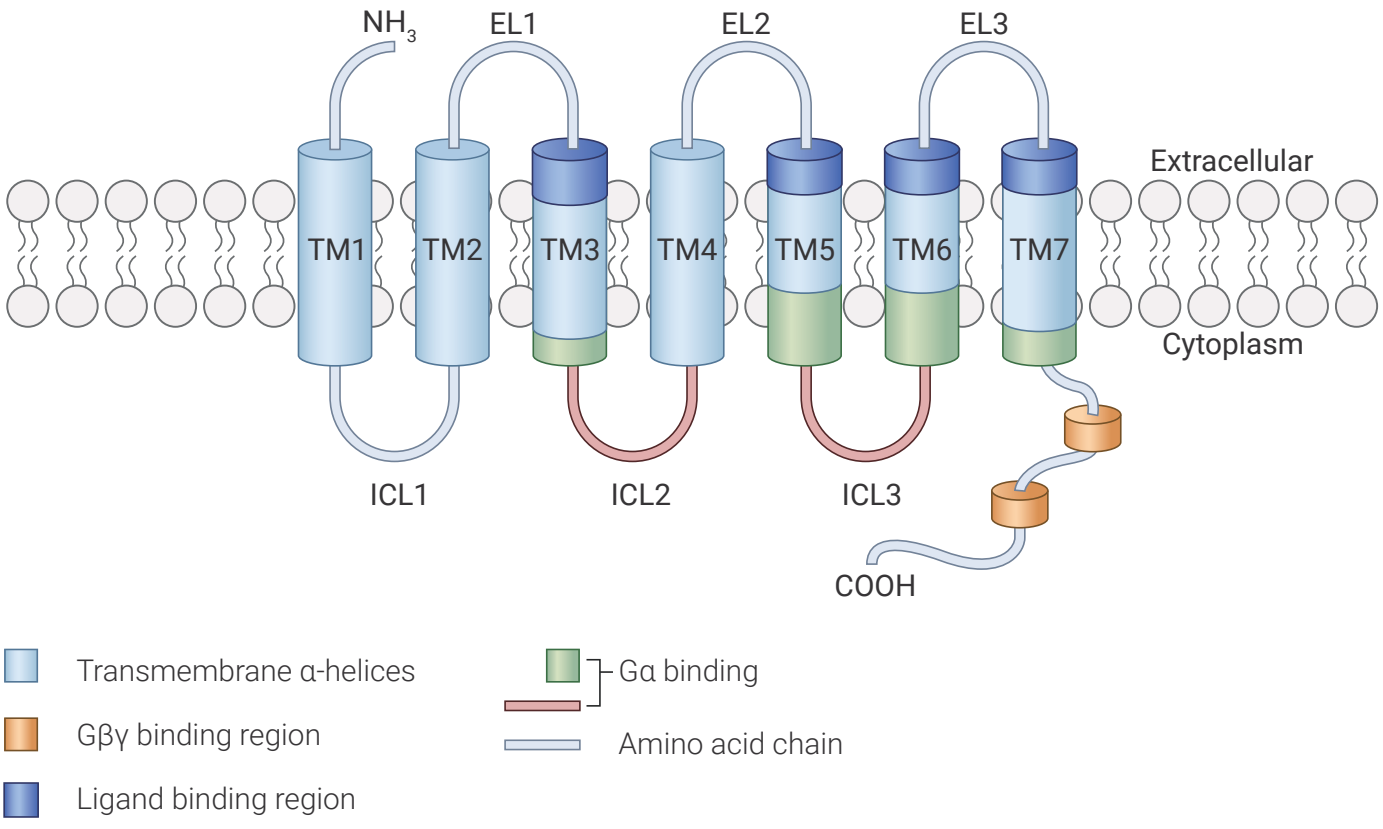
G-protein-coupled receptors (GPCRs) remain one of the most prominent drug targets in the pharmaceutical industry, given their involvement in numerous physiological processes. Recent advancements in GPCR-targeted drug discovery are reshaping the landscape of therapeutics. In 2023 and 2024, there has been significant momentum in the development of next-generation small molecules and biologics targeting previously undrugged GPCRs, especially in areas like oncology and matabolic disease.

Key trends include the exploration of biased agonism, where drugs are designed to selectively activate beneficial signaling pathways while minimizing side effects. Additionally, GPCR oligomerization and allosteric modulation are being closely studied to develop more selective and potent therapies. This is particularly relevant for "orphan" GPCRs, where ligands are not yet well-characterized. Another significant trend is the integration of advanced screening technologies, such as real-time monitoring of intracellular signaling events and high-content screening, to improve early-stage drug discovery.

This thriving research environment highlights the growing opportunities for pharmaceutical innovation, which directly supports the need for specialized services like those offered by ICE Bioscience. Our comprehensive GPCR screening and assay development services are designed to accelerate these innovations, supporting drug discovery teams in identifying and optimizing promising GPCR-targeted therapies.

Why Partner with Us?

- GPCR Assay Development Expertise
- Advanced Binding Solutions
- High-Throughput Functional Screening
- Deep GPCR Dynamics Insight
- Fast and Flexible



Comprehensive GPCR Target Solutions

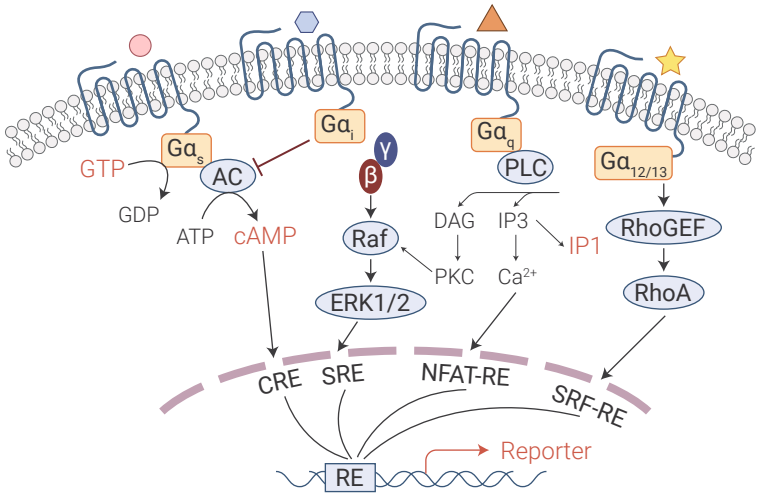
170+ Ready-to-Use Targets							
5-HT1A	A1	CB1	C5AR1	GLP-1R	H3	Rat NOP	IP
5-HT1B	A2A	CB2	D1	Mouse GLP-1R	S1P1	Mouse NOP	PAR1
5-HT1F	A2B	CCR1	D2L	Rat GLP-1R	S1P2	OX1	PAR4
5-HT2A	ALPHA 1A	CCR2	D2S	Dog GLP-1R	S1P3	OX2	PAR2
5-HT2B	ALPHA 1B	CCR4	D3	Monkey GLP-1R	LPA1	P2Y1	SSTR1
5-HT2C	ALPHA 1D	CCR5	D4	Rabbit GLP-1R	LPA3	P2Y2	SSTR2
5-HT4A	ALPHA 2A	CCR6	D5	Pig GLP-1R	MC1	P2Y6	SSTR3
5-HT4B	ALPHA 2B	CCR7	ETA	GLP-2R	MC4	P2Y12	SSTR4
5-HT5A	ALPHA 2C	CCR8	ETB	GIPR	MC5	PTH1R	SSTR5
5-HT7A	BETA 1	CCR9A	Rat ETB	Dog GIPR	MT1	DP1	NK1
5-HT7B	BETA 2	CCR9B	Mouse ETB	Monkey GIPR	MT2	EP1	Rat NK1
M1	AT1	CXCR1	Dog ETB	Mouse GIPR	mGlu2	EP2	Mouse NK1
M2	AT2	CXCR2	FPR1	Rat GIPR	mGlu7	Rat EP2	NK2
M3	APJ	CXCR4	Mouse FPR1	Rabbit GIPR	NPY2R	Mouse EP2	Mouse NK2
M4	TGR5	CCK1	Monkey FPR1	GNRH	DELTA	EP3	Rat NK2
Rat M4	BB1	CCK2	FPR2	Rat GNRH	KAPPA	EP4	NK3
Mouse M4	BB2	GPR17-S	FFAR3(GPR41)	Mouse GNRH	MU	Rat EP4	Rat NK3
Monkey M4	BB3	GPR17-L	GCGR	Rabbit GNRH	Rat MU	Mouse EP4	Mouse NK3
Dog M4	BDKRB1	GPR84	Mouse GCGR	H1	Mouse MU	FP	Rabbit NK3
Rabbit M4	BDKRB2	MRGPX1	Rat GCGR	H2	NOP	TP	TAAR1
M5	GPR40	OXTR	Rat OXTR	MRGPX2	V1A	V1B	V2
CASR	GPR6	GPR3	GPR12	GPR65	GPR52	AMY3	

√ **170+ Ready-to-Use Targets:** We offer over 170 GPCR targets that are fully validated and ready for immediate screening.

√ **50+ Targets in Development:** We are constantly expanding our portfolio, with more than 50 additional GPCR targets currently under development.

√ **40+ GPCR Families:** Our portfolio spans over 40 GPCR families, including key therapeutic targets like serotonin, adrenoceptors, dopamine, and opioid receptors.

√ **Species Diversity:** We provide GPCR assays across multiple species, including human, rat, mouse, monkey, dog, rabbit, and pig, supporting translational research.



GPCR Binding Assays:

- Biophysical Binding Assay
- Radioligand Binding Assay
- Flow Cytometry-Based Binding Assay
- Tag-Lite Binding Assay

GPCR Functional Assays:

- cAMP Assay
- IP1 Assay
- Calcium Flux Assay by FLIPR
- GPCR Reporter Assay
- β-Arrestin Recruitment Assay
- GTPγS Binding Assay
- GPCR Internalization Assay
- pERK/pAKT In-Cell Western Assay
- Chemotaxis Assay

Custom GPCR Cell Line Construction: Flexible Solutions for Tailored Assays

We understand that the ability to develop robust and customized GPCR assays is often more important than simply offering a large catalog of pre-existing assays. As a specialized CRO, our strength lies in our expertise in GPCR cell line construction, which forms the foundation for innovative assay development tailored to our clients' unique research needs.

■ Viral Vector-Mediated Transfection in Mammalian Cells

Our viral transfection system is particularly advantageous for GPCRs that are difficult to transfect or require high expression efficiency. By using a viral vector, we integrate the GPCR gene into mammalian cells, ensuring stable, long-term expression. This approach allows us to generate custom GPCR-expressing clones that can be optimized for various functional assays, including ligand binding and signaling pathway analysis.

■ Plasmid-Based Site-Specific Integration in Mammalian Cells

For applications requiring precise and stable gene insertion at a specific genomic site, we use a plasmid-based system in mammalian cells. This method ensures single-copy gene insertion, resulting in consistent GPCR expression across the cell population. This approach allows us to rapidly generate highly reliable GPCR-expressing cell pools, which serve as a robust platform for developing assays such as high-throughput screening or receptor pharmacology studies.

■ Validation of New GPCR Cell Lines

After constructing new GPCR cell lines, we use a combination of flow cytometry and functional assays to validate the success of the cell line engineering:

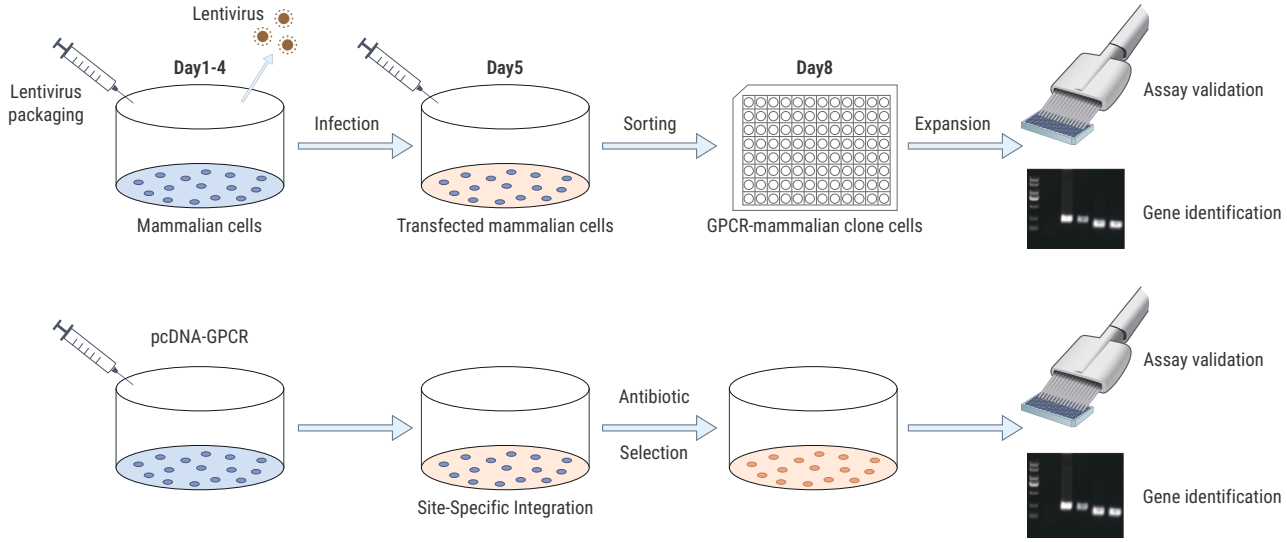


Figure 1. GPCR cell line construction by Lentivirus and Site-Specific Integration system.

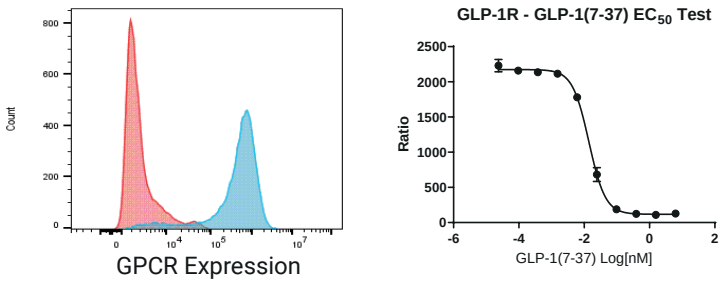
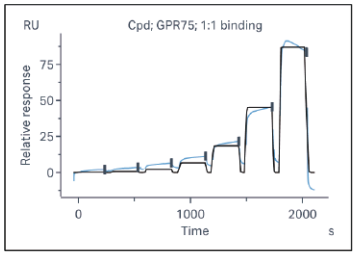


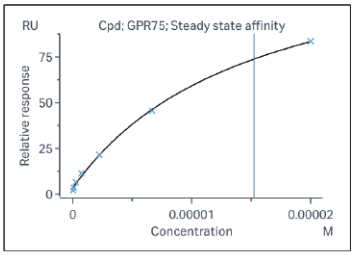
Figure 2. Validation of GPCR cell line by flow cytometry and functional assay.

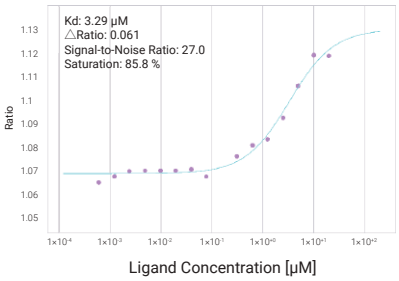
We have collaborated with our partners to successfully obtain 99 types of GPCR membrane preparations, providing a robust foundation for biochemical and biophysical studies in drug discovery.

GPCR Biophysical Binding Assay

Our assay combines the power of Biacore 8K and Dianthus to deliver comprehensive, high-sensitivity binding data. Using Surface Plasmon Resonance (SPR), Biacore 8K provides real-time, label-free measurements of the kinetics and affinity of GPCR-ligand interactions, offering detailed insights into binding dynamics such as association and dissociation rates. On the other hand, Dianthus utilizes Spectral Shift and TRIC (Temperature-Related Intensity Change) technology, enabling rapid, high-throughput binding assays in solution with exceptional sensitivity. This combination allows us to support both detailed kinetic studies and high-throughput screening.







0.02743 0.0823 0.24691 0.74074 2.22222 6.66667 20µM

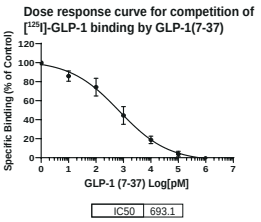
1:1 binding ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Quality Kinetics Chi² (RU²)	U-value	Steady state affinity KD (M)	Rmax (RU)
1.7e+04	2.9e-01	1.7e-05	162.2	1.6e+01	20	1.5e-05	141.0

Ligand	Evaluator	KD	Signal-to-Noise Ratio
Cpd	Spectral Shift	3.29 µM	27.0

Figure 3. Biophysical binding assays using His-GPR75-M protein were performed with Biacore 8K (96-well plate) for SPR analysis and Dianthus (384-well plate) for Spectral Shift, providing KD and signal-to-noise data.

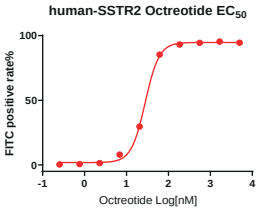
GPCR Radioligand Binding Assay

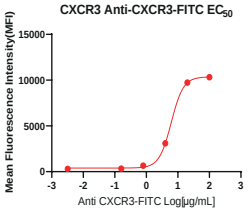
It is a classic method for quantifying the binding affinity of ligands to GPCRs using radioactively labeled ligands. This assay provides highly sensitive and precise measurements of ligand-receptor interactions. By tracking the binding of a radiolabeled ligand, we can determine key properties like binding affinity and competitive displacement by test compounds.



GPCR Flow Cytometry-Based Binding Assay

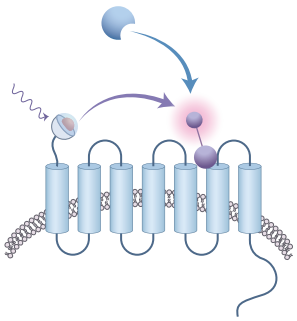
The assay is an advanced tool designed for the quantitative analysis of receptor-ligand interactions in living cells. This assay is valuable for studying cell surface GPCRs, where fluorescently labeled ligands or antibodies can detect and quantify receptor binding in real time.

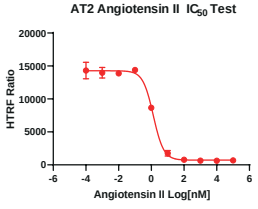




GPCR Cell Based HTRF Tag-Lite Binding Assay

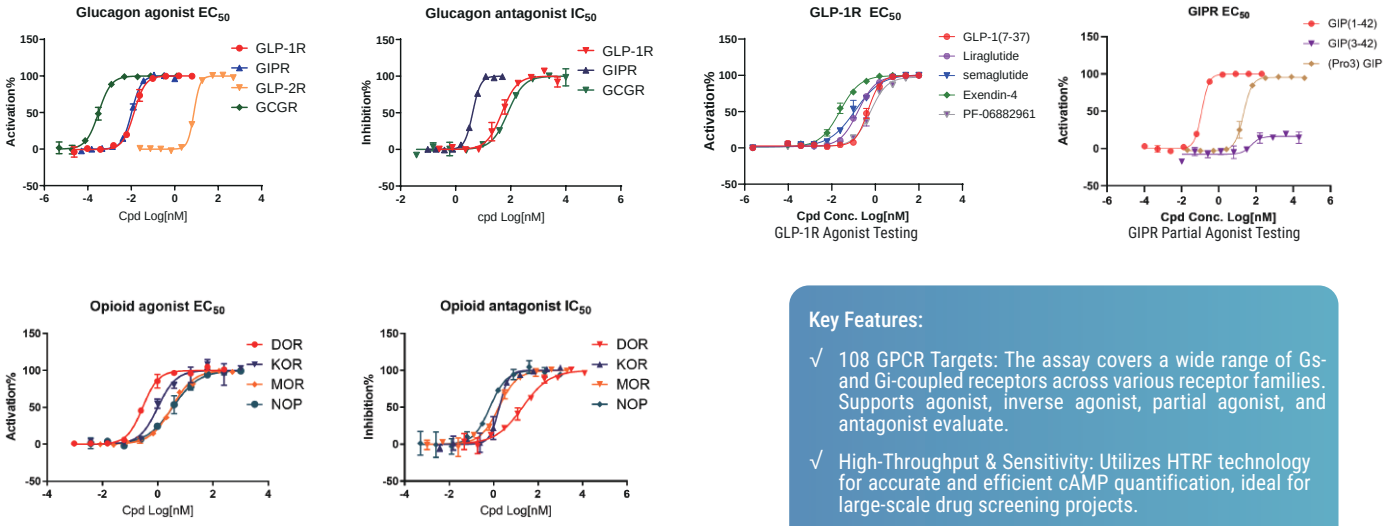
The assay utilizes SNAP-tagged GPCRs, which are covalently labeled with a fluorescent marker, allowing for the study of receptor-ligand interactions in live cells. This system operates via a competitive binding format, and the resulting signal change enables quantification of binding affinity.





GPCR Cell Based HTRF cAMP Assay

Detecting cAMP in GPCR signaling involves measuring the levels of cyclic AMP produced when Gs/Gi-coupled GPCRs activate/inhibit adenylyl cyclase, which converts ATP to cAMP, serving as a key second messenger in downstream signaling pathways. Our GPCR Cell-Based HTRF cAMP Assay is specifically designed to measure cAMP levels, providing a key readout for G protein-dependent signaling through Gs or Gi-coupled GPCRs. This assay directly captures the primary signal transduction event following receptor activation.



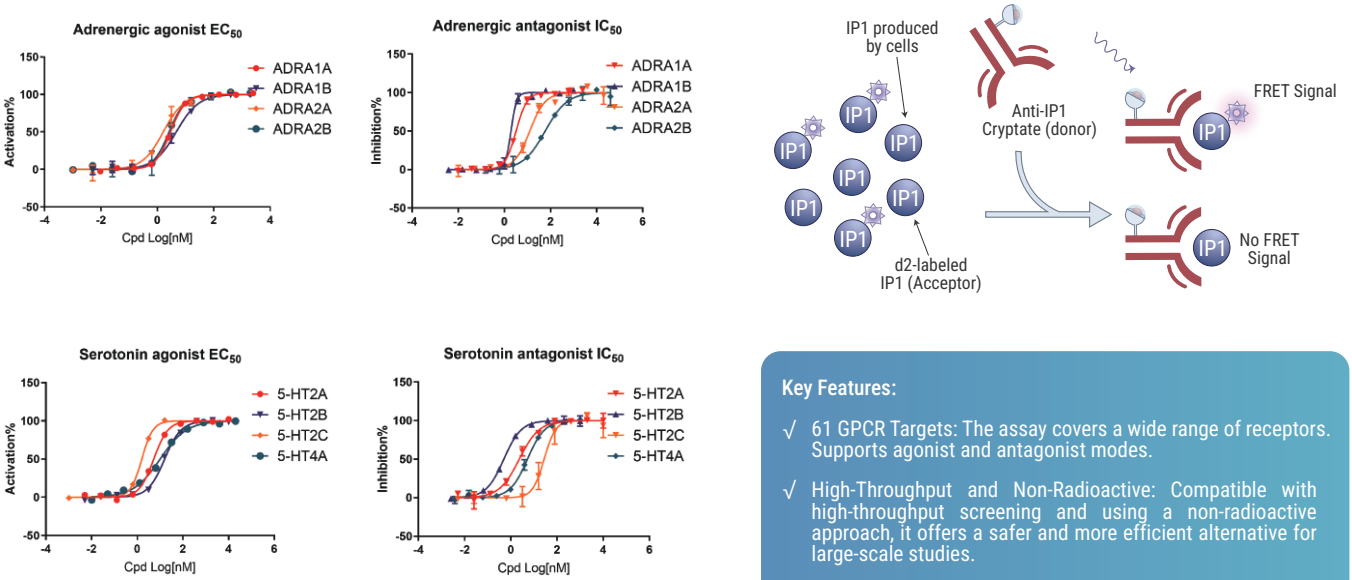
Glucagon and Opioid Receptor Testing in Agonist/Antagonist Mode

Key Features:

- √ 108 GPCR Targets: The assay covers a wide range of Gs- and Gi-coupled receptors across various receptor families. Supports agonist, inverse agonist, partial agonist, and antagonist evaluate.
- √ High-Throughput & Sensitivity: Utilizes HTRF technology for accurate and efficient cAMP quantification, ideal for large-scale drug screening projects.
- √ TAT: 5-6 days

GPCR Cell Based HTRF IP1 Assay

This assay is designed to measure the buildup of inositol monophosphate (IP1), it's especially valuable for studying G protein-coupled receptors (GPCRs) that activate through the Gq pathway. In this process, the enzyme phospholipase C (PLC) is triggered, leading to the production of inositol trisphosphate (IP3), which is quickly converted into IP1. This assay can directly monitor the efficiency of drug and its compatibility with high-throughput screening systems makes it an ideal technique for screening potential drugs for Gq-coupled GPCRs.



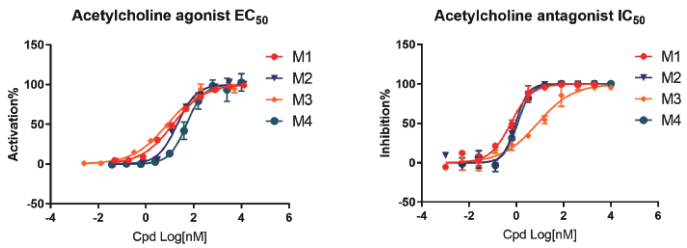
IP1 Agonist and Antagonist Assays

Key Features:

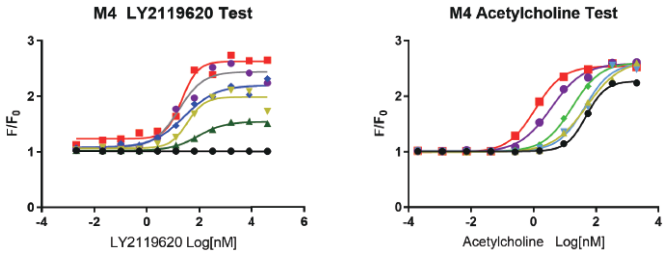
- √ 61 GPCR Targets: The assay covers a wide range of receptors. Supports agonist and antagonist modes.
- √ High-Throughput and Non-Radioactive: Compatible with high-throughput screening and using a non-radioactive approach, it offers a safer and more efficient alternative for large-scale studies.
- √ TAT: 5-6 days

GPCR Cell Based Calcium Flux Assay

Our GPCR Cell-Based Calcium Flux Assay utilizes the FLIPR (Fluorescence Imaging Plate Reader) system to measure changes in intracellular calcium levels, a key indicator of Gq protein-dependent signaling. This assay is ideal for studying GPCRs that couple to Gq proteins, which activate phospholipase C (PLC), leading to the release of calcium from intracellular stores. It is well-suited to detect PAM activity for Gq-coupled GPCRs. The calcium flux assay offers real-time, dynamic monitoring of GPCR activation by detecting rapid changes in calcium concentrations.



Cell Based Calcium Flux Assay



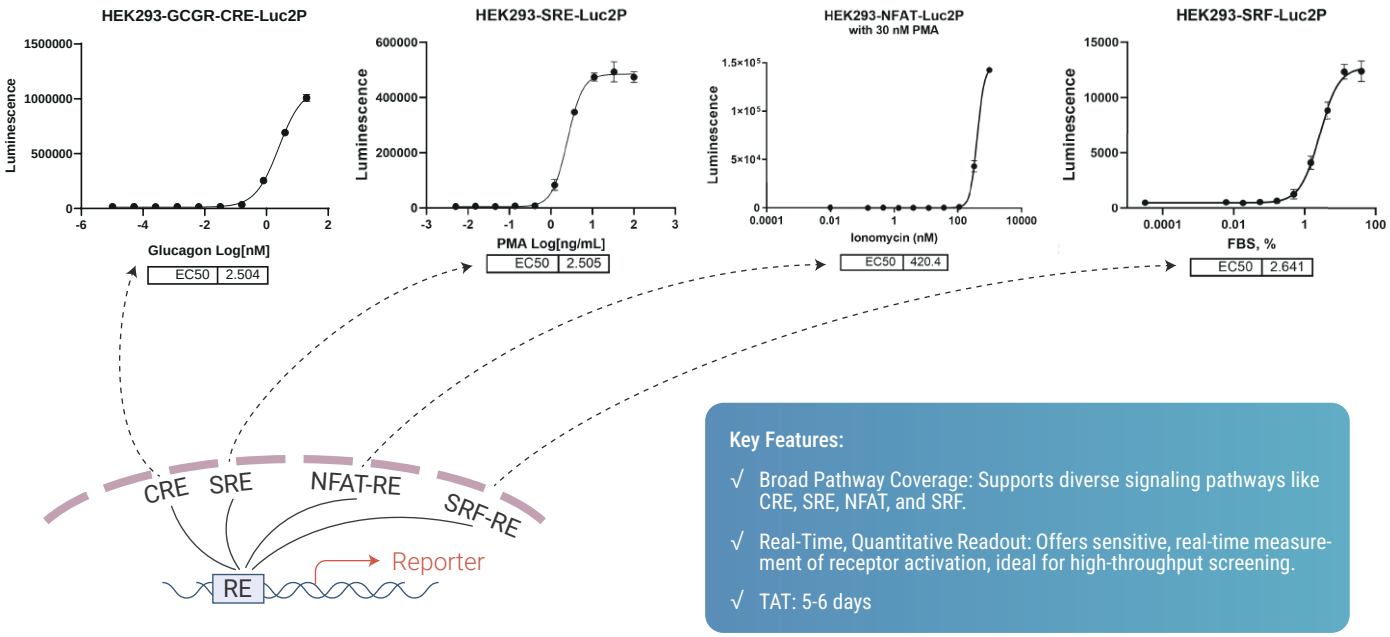
M4 PAM Calcium Flux Assay

Key Features:

- √ 50+ GPCR Targets: Supports agonist, antagonist and PAM modes. ELSE?
- √ It enables real-time detection of Gq-coupled GPCR activation while supporting high-throughput screening.
- √ TAT: 5-6 days

GPCR Cell Based Reporter Assay

The assay measures GPCR activation by utilizing reporter genes like luciferase that are linked to specific signaling pathways. Upon GPCR activation, these pathways trigger the expression of the reporter, producing a measurable signal. This assay is ideal for high-throughput screening of GPCR agonists, antagonists, or modulators, offering a real-time and quantitative readout of receptor activity. It is particularly useful for studying GPCR-mediated transcriptional responses.

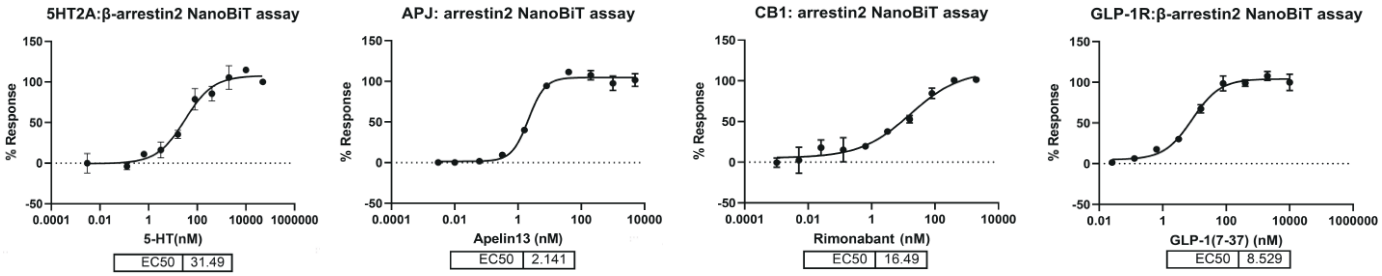


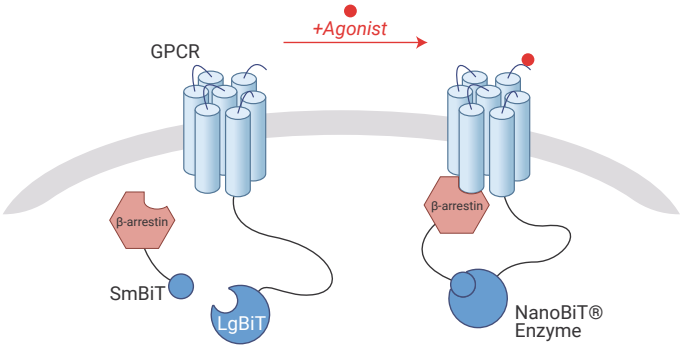
Key Features:

- √ Broad Pathway Coverage: Supports diverse signaling pathways like CRE, SRE, NFAT, and SRF.
- √ Real-Time, Quantitative Readout: Offers sensitive, real-time measurement of receptor activation, ideal for high-throughput screening.
- √ TAT: 5-6 days

GPCR β-Arrestin Recruitment Assay

β-arrestin recruitment is the process where β-arrestin binds to a phosphorylated GPCR after activation, blocking further G-protein signaling and facilitating receptor desensitization, internalization, and initiation of alternative signaling pathways such as MAPK/ERK. Unlike traditional functional assays that measure second messengers (e.g., cAMP or calcium flux), this assay provides insights into β-arrestin-dependent signaling. Upon β-arrestin recruitment, the system generates a luminescent signal through the reconstitution of luciferase subunits, offering real-time, low-background detection.



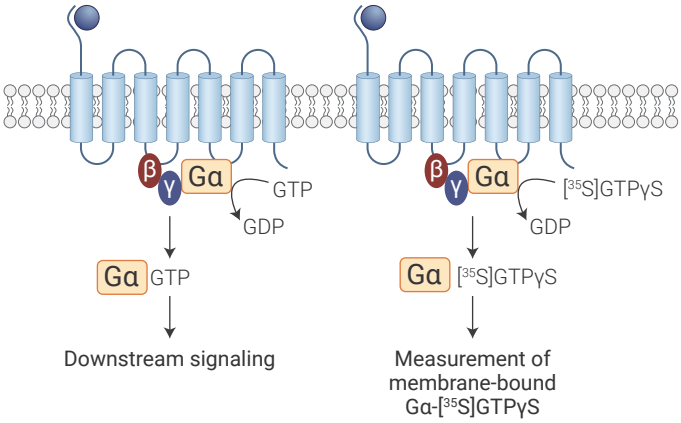


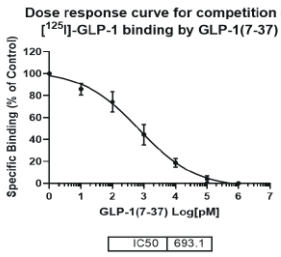
Key Features:

- √ 21 Ready-to-Test Targets: We provide 21 validated GPCR targets, allowing for immediate testing of β-arrestin recruitment.
- √ Novel Assay Development Support: Our platform can be adapted to develop new GPCR-based PPI assays in about 1 month.
- √ TAT: 1-2 weeks

GPCR GTPγS Binding Assay

GTPγS, a non-hydrolyzable analog of GTP, binds to the G-protein, allowing for the direct assessment of GPCR-mediated G-protein activation without further signaling turnover. The GTPγS Binding Assay is a widely used functional assay that measures the activation of G-proteins upon GPCR stimulation. It provides real-time data on receptor functionality rather than just binding affinity.



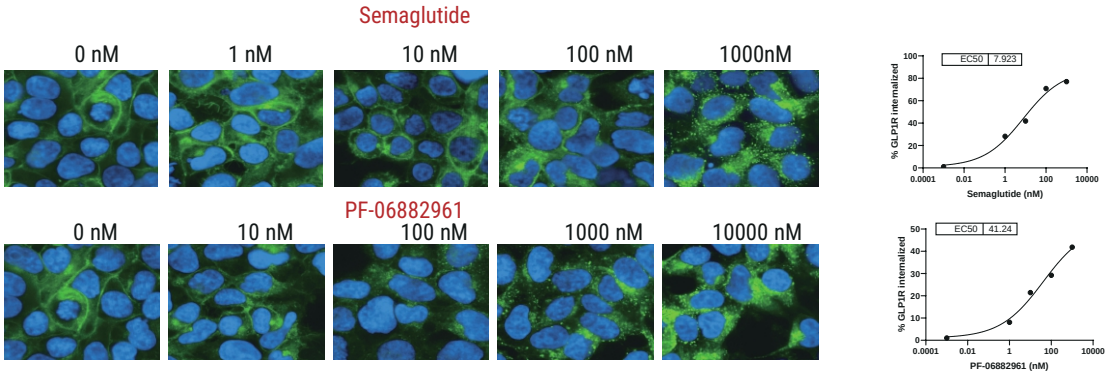


Key Features:

- √ Direct G-Protein Activation Readout: Measures GTPγS binding, offering precise insight into GPCR-mediated G-protein activation.
- √ Ideal for Ligand Bias Studies: Excellent for distinguishing G-protein signaling bias over other pathways, like β-arrestin.
- √ High Sensitivity for Agonist Potency: Detects subtle differences in agonist potency and efficacy, crucial for characterizing partial and inverse agonists.

GPCR Internalization Assay

GPCR internalization is the process by which activated and phosphorylated GPCRs are removed from the cell surface and internalized into the cell, typically via clathrin-mediated endocytosis, to regulate receptor desensitization, resensitization, or degradation. High-content imaging allows for real-time, quantitative measurement of receptor internalization following ligand binding. This assay provides detailed visualization and analysis at the cellular level, making it ideal for drug discovery applications.



The internalization of GLP-1R in response to the agonists Semaglutide and PF-06882961, as visualized by high-content imaging. Internalization is more pronounced at higher concentrations for both compounds.

Assay Format	Semaglutide (EC50)	PF-06882961 (EC50)
HTRF cAMP Assay	0.1176 nM	0.5963 nM
Internalization Assay	7.923 nM	41.24 nM

The table indicates that Semaglutide is more potent in both activating the receptor (as measured by cAMP production) and inducing receptor internalization.

Key Features:

- √ Quantitative Visualization
- √ High-Throughput Capability
- √ Real-Time Internalization Tracking
- √ High content image

Why Measure GPCR Signaling Bias?

GPCR signaling bias, also known as ligand bias, refers to the ability of a ligand (e.g., drug or compound) to preferentially activate specific downstream signaling pathways over others when bound to a GPCR. GPCRs typically signal through multiple pathways, such as G-protein-dependent signaling (e.g., cAMP, calcium) and β-arrestin-mediated signaling (involved in receptor desensitization and internalization). A biased ligand selectively activates one of these pathways over the other, rather than triggering all pathways equally.

- **Increased Therapeutic Efficacy:** Biased ligands can be designed to selectively activate beneficial signaling pathways while minimizing activation of pathways that lead to side effects. For example, in opioid receptors, ligands that favor G-protein signaling over β-arrestin pathways may provide pain relief while reducing adverse effects like respiratory depression.

- **Reduced Side Effects:** Many GPCR-related drugs produce side effects because they activate multiple signaling pathways, some of which lead to unwanted biological responses. By targeting specific pathways (i.e., biased agonism), drugs can be made more selective, reducing the risk of adverse effects and improving patient safety.

Target	Assay	EC50/IC50 (nM)
MOR	cAMP HTRF Assay	IC50=1.722
	β-arrestin Recruitment Assay	IC50=4.122
GLP-1R	cAMP HTRF Assay	EC50=0.730
	β-arrestin Recruitment Assay	EC50=8.529
5-HT2A	Calcium Flux Assay	EC50=1.819
	β-arrestin Recruitment Assay	EC50=31.49

- **Tailored Therapeutic Profiles:** Biased ligands can offer a more refined pharmacological response tailored to specific therapeutic needs. This is especially valuable in complex diseases where modulating one pathway might be beneficial while avoiding others that could worsen the condition.

? Interested in learning more about our chemotaxis assays, or the pERK/pAKT ICW and pERK HTRF assays? Feel free to contact us for detailed information!

Which GPCR Assay Fits Your Needs?

Assay Type	Assay	Readout	Ideal Applications	Key Advantages
Binding Assays	Radioligand Binding Assay	Ligand-Receptor Binding	High-affinity ligand binding; drug screening	High sensitivity for low-concentration ligands; quantitative data with precision; proven method for decades
	Biophysical Binding Assay (SPR, Spectral Shift, TRIC)	Kinetics & Affinity (ka, kd, KD)	Real-time ligand-receptor binding studies;drug screening and optimization	SPR provides real-time kinetic data (ka, kd, KD); Spectral Shift and TRIC offer label-free detection for binding in solution; ideal for screening small molecules or fragments
	Tag-Lite Binding Assay	Ligand Binding (Live Cells)	High-throughput screening, GPCR drug discovery	Homogeneous format; no wash steps needed; ideal for large compound libraries and rapid screening
	Flow Cytometry-Based Binding Assay	Surface Receptor Binding	Single-cell level receptor-ligand binding analysis	Allows analysis of receptor expression and binding on a per-cell basis; supports complex cellular environments
Functional Assays	cAMP Assay	Gs/Gi Protein Signaling	Evaluating cAMP production via GPCR activation	Quantifies changes in intracellular cAMP levels; real-time measurements; sensitive for Gs/Gi pathway studies
	IP1 Assay	Gq Protein Signaling	Studying GPCRs that trigger phosphoinositide turnover	Specific for Gq-coupled receptors; provides quantifiable readout of IP1 accumulation, correlating to receptor activity
	Calcium Flux Assay (FLIPR)	Gq Protein Signaling	Real-time detection of calcium mobilization; rapid signaling	High-throughput, sensitive; measures fast transient signals; ideal for evaluating calcium-based GPCR signaling
	GPCR Reporter Assay	Transcriptional Responses	Assessing transcription-based GPCR activity (e.g., CRE-Luc)	High throughput; various reporter formats (luciferase, fluorescence); great for monitoring long-term effects
	β-Arrestin Recruitment Assay	β-Arrestin Binding	Monitoring receptor desensitization and trafficking	Measures recruitment of β-arrestin; ideal for studying receptor internalization and biased signaling
	GTPγS Binding Assay	G Protein Activation	Direct measurement of G-protein activity	Quantitative; ideal for screening agonists, antagonists, and inverse agonists; no secondary messengers needed
	GPCR Internalization Assay	Receptor Internalization	Monitoring receptor endocytosis post-ligand binding	High-content imaging; tracks internalization in real-time; useful for evaluating receptor regulation dynamics
	pERK/pAKT ICW Assay	Downstream MAPK/PI3K Pathways	Assessing phosphorylation of ERK/AKT as downstream effect	Direct, quantitative readout of pathway activation (ERK, AKT); correlates GPCR activation with cell proliferation or survival
	Chemotaxis Assay	Cell Migration	Evaluating cell movement in response to chemokines	Real-time quantification of migration; useful for studying GPCRs involved in immune response and cancer metastasis

GPR75 has become a highly promising target in drug discovery, especially for obesity treatment. Recent large-scale genetic studies by Regeneron and collaborators discovered that people with loss-of-function mutations in the GPR75 gene had a 54% lower risk of obesity and tended to weigh significantly less. This breakthrough highlights the potential of GPR75 as a novel therapeutic target to combat obesity, a growing global health challenge expected to affect over a billion people by 2030.

ICE Bioscience offers a comprehensive suite of GPR75 biophysical and functional assays for small molecule screening and characterization. Available Assays:

GPR75 Biophysical Binding Assay (see Page 4)

- ◇ Purpose: Real-time binding kinetics analysis of small molecules and protein ligands using SPR and Spectral Shift.
- ◇ Technology: Provides detailed kinetic profiles including association (ka) and dissociation (kd) rates, steady-state affinity (KD), and Rmax. This assay is ideal for understanding the binding interactions of small molecules and peptides with GPR75.
- ◇ Application: Suitable for lead optimization and in-depth mechanistic studies of receptor-ligand interactions.

GPR75-SRE-Luc Reporter Assay

- ◇ Purpose: Screening of agonists and inverse agonists using HEK293T-GPR75-SRE-Luc cells.
- ◇ Format: 384-well plate; high-throughput and automated with a 3-day turnaround.
- ◇ Protocol: Cells are seeded, followed by compound addition using Echo655, incubation, and luminescence detection via BMG FSX. The assay is ideal for HTS and can be used to rapidly evaluate compound efficacy.
- ◇ Application: Suitable for lead identification and characterization in early drug discovery. A 10K compound library is available, with a lead time of 1 month for screening.

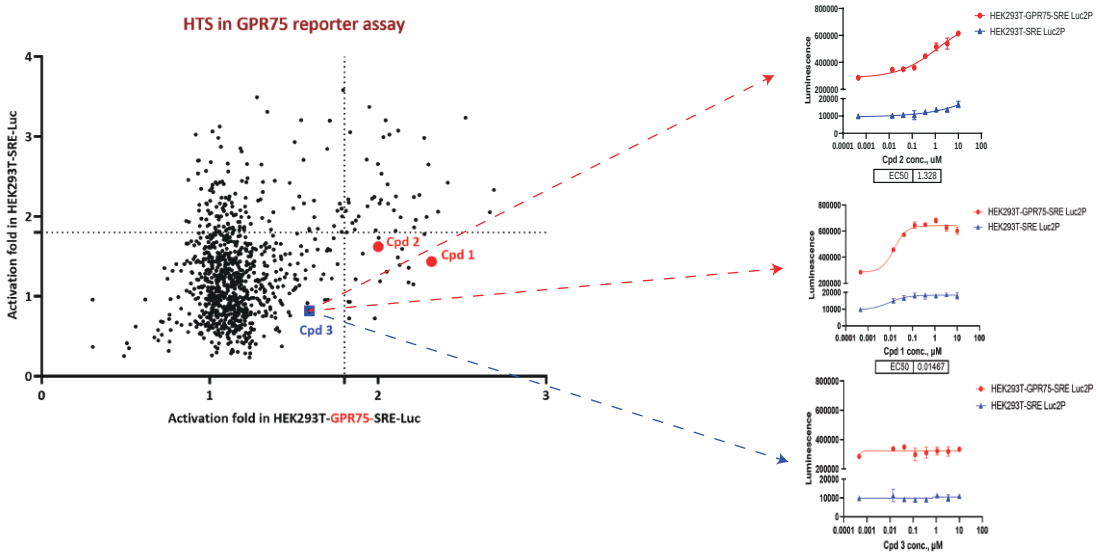
Under Development

GPR75-EGFP Internalization Assay

- ◇ Purpose: Validates GPCR internalization through EGFP-tagged GPR75 to monitor receptor trafficking post-ligand binding.
- ◇ Application: Used for studying receptor internalization and desensitization, particularly in response to small molecules and peptides.

GPR75-β-Arrestin 2 Recruitment Assay

- ◇ Purpose: Measures β-arrestin recruitment, providing insights into GPCR desensitization and internalization following activation. This assay supports antagonist and agonist testing.
- ◇ Format: Real-time luminescence-based detection, offering high sensitivity and low background noise.



Interested in Understanding HFD/STZ and HFD Induction Models? Contact us for more information and tailored solutions!

Induction	HFD/STZ (IP)	HFD
Animal	C57BL/6, 4 weeks	C57BL/6, 8 weeks
Criteria	Blood sugar (BS) ≥16.7mM	BW，Blood glucose，IGTT
Treatment	10-12 weeks	16-20 weeks
Routes	PO,IP,IV,SC	PO,IP,IV,SC
Examination	BS, FBS, BW, GTT, AST, ALT etc.	PO,IP,IV,SC
Optional	Pathology，biomarker，PK/PD，in vivo MOA	

ICE Bioscience

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.

To learn more, please visit: en.ice-biosci.com