



GPCRs: THE PATHWAY TO DISCOVERY

Complete Solutions for Drug Discovery

Take Aim at the Perfect Target

The largest family of cell-surface membrane receptors and one of the most intensely studied drug targets, G protein-coupled receptors, or GPCRs, hold out tremendous promise for scientists. In fact, therapies that target GPCRs include ligands from all pharmacological classes (agonists, antagonists, inverse agonists, allosteric modulators) used to treat diseases in every major organ system and for several therapeutic areas, including cardiovascular diseases, neurosciences, respiratory ailments, metabolic disorders, oncology, and rare diseases. What's more, their surface location makes them accessible to a whole host of druglike molecules.

How important are GPCRs to drug discovery? It's estimated that up to one-third of all marketed therapeutics act by binding to GPCRs.

We know the importance of furthering GPCR research – and we answer the call with instruments, reagents, and tools and technologies for all aspect of research into today's most promising drug target.



**eBook: Understanding
GPCRs**



Guide: Pharmacology

Gs, Gi, and Gq Signaling

G proteins are classified as Gi, Gs, or Gq and signal through different pathways. Gq proteins rely on phospholipase C (PLC) enzymes, while Gs and Gi proteins stimulate and inhibit adenylate cyclase (AC) and act on the amount of cytosolic cyclic AMP (cAMP).

With GPCR signaling, the best assay conditions depend on the GPCR coupling (Gq) efficacy and the pharmacological characteristics of compounds being studied and cell lines used.

The metabolic inositol phosphate cascade often results from regulation of PLC- β associated with the G α_q subunit of heterotrimeric G protein. Measuring an important mediator such as D-myo-inositol 1-phosphate (IP1) can be used as a surrogate for IP3. G α_q -coupled GPCRs act to stimulate the activity of PLC- β , resulting in an increase in cellular IP1.

cAMP is one of the most important GPCR intracellular mediators, often resulting from regulation of AC by the G α_s subunit of heterotrimeric G proteins. G α_s -coupled GPCRs act to stimulate the activity of AC, resulting in an increase in cellular cAMP. G α_i -coupled GPCRs lead to a negative regulation of AC, and to a decrease in cAMP production.



Guides: Optimizing Gs and Gi Agonist and Antagonist Assays



Guides: Master IP-One Assays

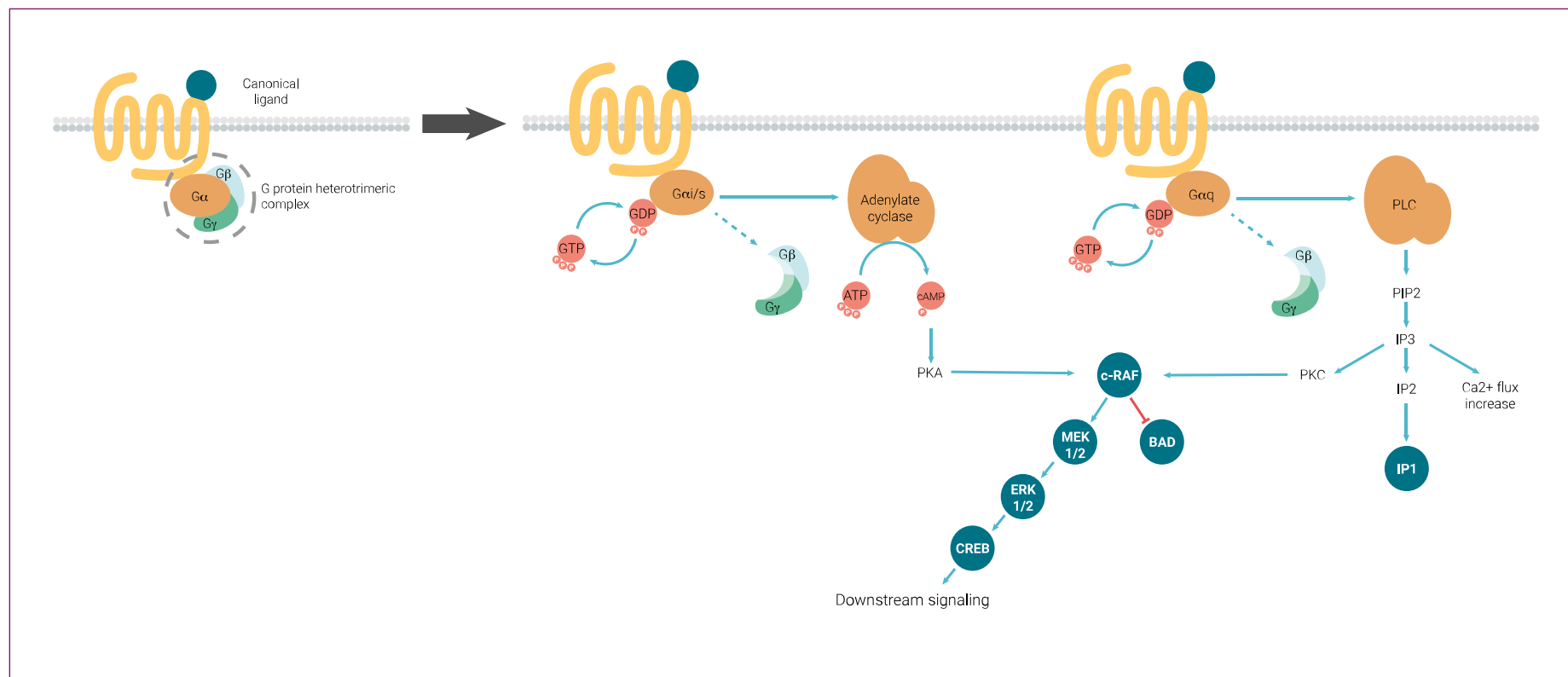


White Paper: IP-One



White Paper: GPCR Second Messenger Data

Gs, Gi, and Gq Signaling



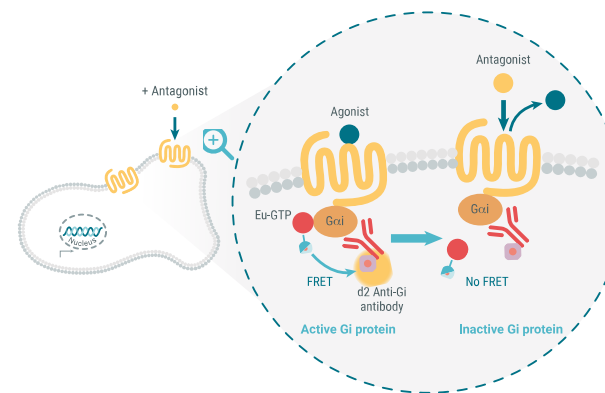
G-Protein Signaling – Gs, Gi, and GQ are types of G-protein α subunits.

Gs, Gi, and Gq Signaling: GTP γ S – GTP Binding Assay

Guanosine 5'- (trihydrogen-diphosphate), or GTP γ S, is a nonhydrolyzable analog of GTP and exhibits similar physiological behavior, such as the stimulation of guanine nucleotide-binding proteins.

The GTP binding assay measures G-protein activation levels following agonist occupation of a GPCR by determining the binding of the GTP to the G subunit α . The assay measures a functional consequence of receptor occupancy at one of the first receptor-mediated events.

This simple assay provides a better understanding of contemporary pharmacological issues, including the role of accessory proteins in signaling, GPCR-receptor-constitutive activity, and agonist-specific signaling.



Activation of GPCR leads to GDP/GTP nucleotide exchange on the G α protein subunit and binding of the Eu-GTP analog. Detection is enabled by the addition of the d2 anti-Gi antibody. An antagonist addition competes with the agonist binding in the orthosteric site of GPCR and inactivates the G protein. Eu-GTP dissociates, leading to FRET signal extinction.



Guides: Optimize GTP Binding Assays



Application Note: Identification and Characterization of GPCR Compounds



Application Note: Upstream and Downstream GPCR Readouts



Application Note: Pharmacological Validation of a Panel of GPCRs

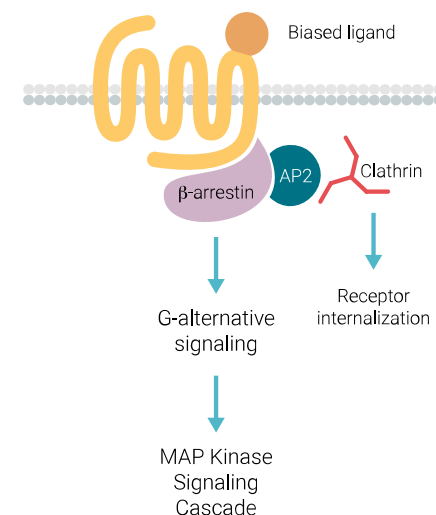
β-Arrestin Signaling

Arrestins are a small family of proteins that regulate signal transduction at G protein–coupled receptors. β-arrestins play an essential role in many physiological and pathological processes, including desensitization, internalization and sequestration, and trafficking of GPCR.

β-arrestin 1 and β-arrestin 2 are mediators of desensitization and internalization of GPCRs and are widely distributed in various tissues and cells. Both accumulate in the cytoplasm of cells, but β-arrestin 1 also accumulates in the nucleus.

These proteins act as scaffold proteins and are associated with certain components of the MAPK cascade and downstream targets of various GPCRs, including ERK and AKT.

Due to their ability to inhibit broad G-protein signals and activate more straightforward transduction cascades, β-arrestins offer attractive opportunities to develop therapeutics less susceptible to carrying adverse effects than usual GPCR-targeted drugs.



β-arrestin pathway, and arrestins binding clathrin and its adaptor AP2 – key components of the internalization of GPCR machinery



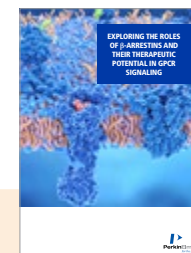
Technical Note: Key Success Tips to Performing B-Arr2 Recruitment Assays



Application Note: Inducing β-Arrestin 2 Recruitment

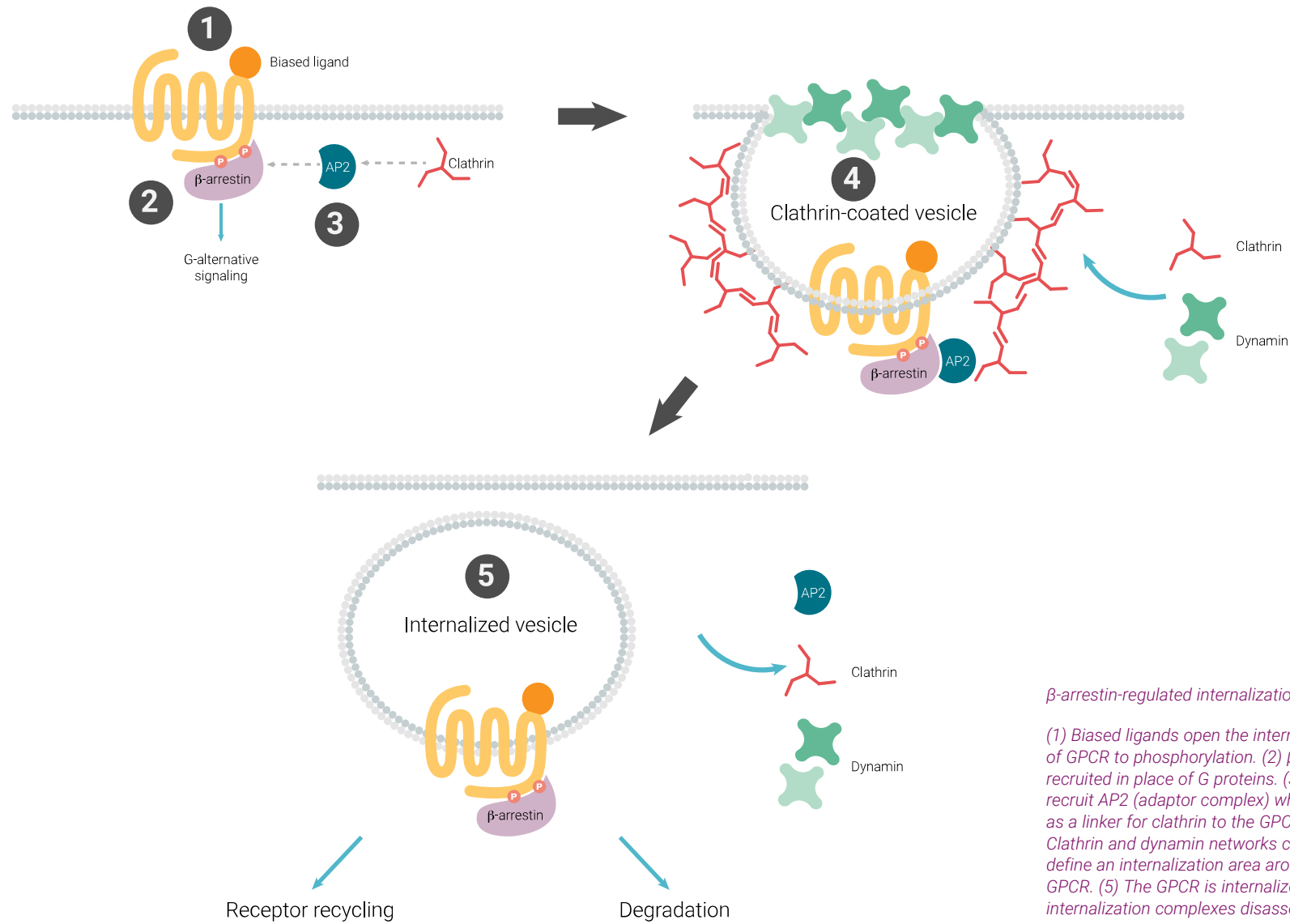


Application Note: Tools for β-Arrestin Monitoring in Various Cells



eBook: Exploring the Roles of β-Arrestins and Their Therapeutic Potential in GPCR Signaling

β-Arrestin Signaling



β-arrestin-regulated internalization of GPCR

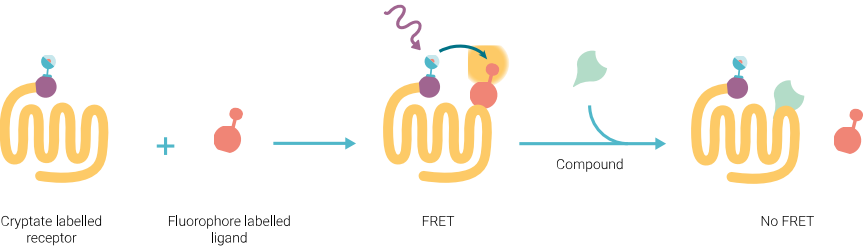
(1) Biased ligands open the internal segment of GPCR to phosphorylation. (2) β-arrestin is recruited in place of G proteins. (3) β-Arrestin recruit AP2 (adaptor complex) which acts as a linker for clathrin to the GPCR. (4) Clathrin and dynamin networks coat and define an internalization area around the GPCR. (5) The GPCR is internalized and the internalization complexes disassemble.

Receptor Binding

Nonradioactive Tag-lite® binding assays offer straightforward add-and-read protocols to help you characterize the binding properties of compounds, regardless of their chemical structures or pharmacological properties

Eighteen Tag-lite binding assays have been validated, with custom binding solutions available. Tag-lite offers freedom and flexibility to build your assays from target construction to assay development.

To determine K_d , the fluorescent ligand is titrated into a solution containing a fixed number of labeled cells and then incubated to equilibrium. To determine K_i , the compound is titrated into a solution with a fixed concentration of fluorescent ligand and a fixed quantity of cells. At equilibrium, the fraction of labeled ligand bound to the receptor is proportional to the recorded FRET signal. Binding affinities are calculated from this resulting signal.



Tag-lite assay principle



Application Note:
Determination of K_{on} and K_{off}
Rate Constants with Tag-Lite



Poster: Multidimensional
Assays for GPCRs

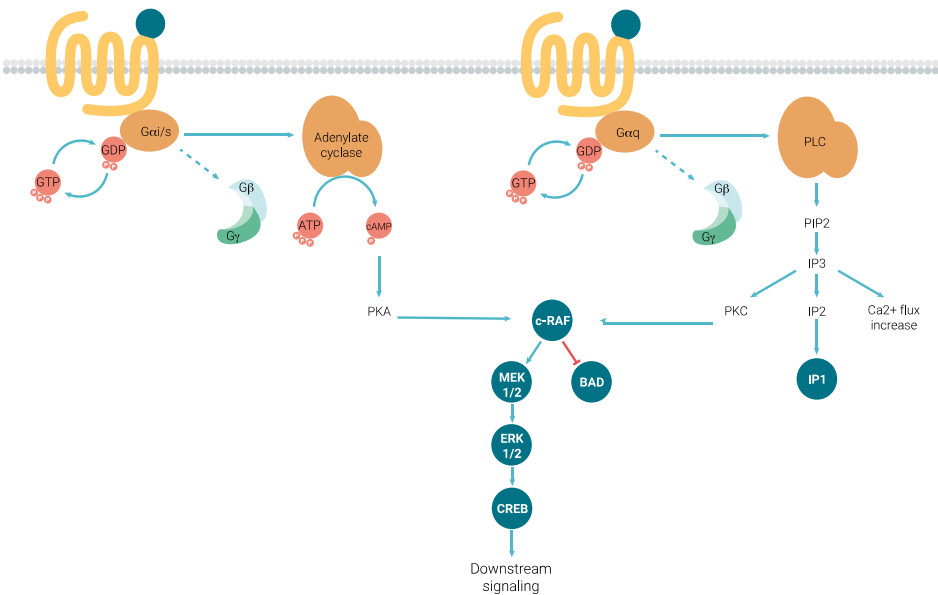


Flyer: Tag-lite Binding Assay

Phosphorylation Pathways

When GPCRs become activated, they transduce a signal first through G proteins and then a second messenger. The third part of that transduction signal chain involves phosphoprotein pathways that lead to transcription factors and changes in gene expression.

While all GPCRs differ in their specific signaling pathways, phosphatases are always involved, serving as triggers and promoters for phosphorylation cascades down to the nucleus. The MAP kinase pathway is shared by the three GPCR types and offers additional opportunities for GPCR activity monitoring, with results in the areas of cell survival, proliferation, differentiation, and metabolism.



GPCRs are at the head of many phosphorylation cascades resulting from phosphatases bound to their second messengers



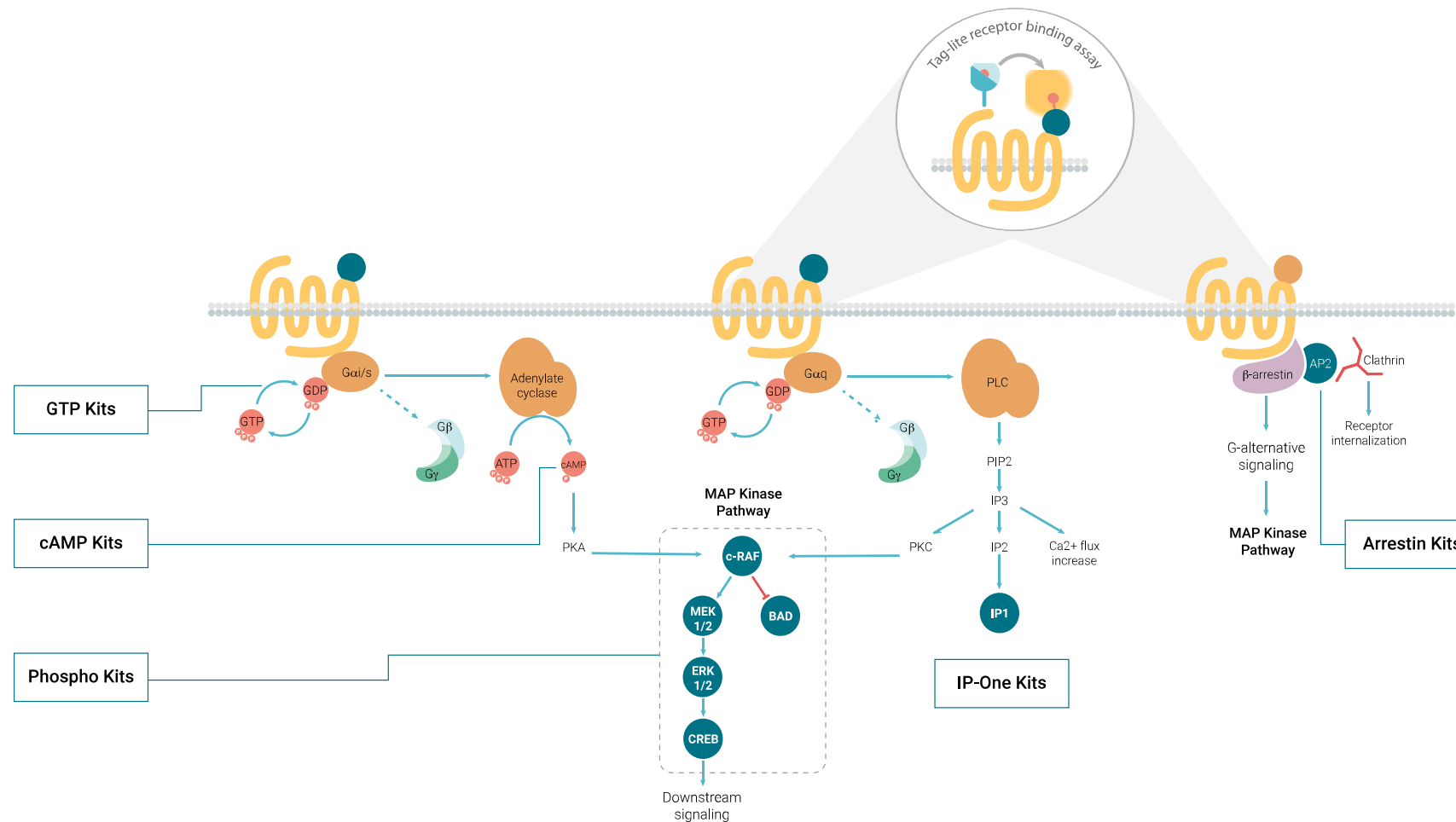
Guide: Cell Signaling Experiments



Application Note: GPCR-Mediated ERK1/2 Phosphorylation

GPCR Reagents

Our assays address the whole GPCR activation pathway, covering all categories of binding and signaling to support your research. Our kits are available in multiple formats, such as [HTRE®](#) and [AlphaLISA®](#), providing quick and accurate methods that are easily incorporated into your laboratory workflow.



GPCR Reagents Portfolio

| PHOSPHORYLATION ASSAYS | TECHNOLOGY |
|---|--|
| AKT total | HTRF |
| AKT phospho S/T | HTRF |
| AKT phospho S473 | HTRF, ASSF |
| AKT phospho T308 | HTRF, MPSU, DELFIA®, LANCE® |
| AKT/PKB | LANCE <i>Ultra</i> |
| AKT1 total | HTRF, ALSU, ASSF, LANCE <i>Ultra</i> , LANCE |
| AKT1 phospho S308 | ALSU, LANCE, LANCE <i>Ultra</i> |
| AKT1 phospho S473 | HTRF, ALSU, ASSF, LANCE <i>Ultra</i> |
| AKT1 phospho T308 | ASSF, LANCE <i>Ultra</i> |
| AKT2 total | HTRF |
| AKT2 phospho S473 | HTRF |
| AKT3 total | HTRF |
| AKT3 phospho S473 | HTRF |
| AKT1/2/3 total | ALSU, MPSU, ASSF |
| AKT1/2/3 phospho S473 | ALSU, LANCE <i>Ultra</i> , LANCE |
| AKT1/2/3 phospho T308 | ALSU, TBSU, ASSF, LANCE <i>Ultra</i> |
| AKT1/2/3 phospho S473 and AKT1 total | MPSU |
| AKT1/2/3 phospho S473 and ERK 1/2 phospho T202/Y204 | MPSU |
| AMPK total | HTRF |
| AMPK phospho T172 | HTRF, ALSU, LANCE <i>Ultra</i> |
| AMPKa1/2 | ALSU |
| AMPKa phospho T172 | ALSU, LANCE <i>Ultra</i> |
| AMPKa1/2 phospho T172 and total | MPSU |
| BAD phospho S112 | HTRF, ASSF |
| BAD phospho S136 | ASSF |
| CREB total | HTRF, ALSU, LANCE <i>Ultra</i> |
| CREB phospho S133 | HTRF, ALSU, TBSU, ASSF, LANCE <i>Ultra</i> |
| ERK total | HTRF, ASSF, Alpha CETSA, MPSU |
| ERK phospho T202/Y204 | HTRF, ALSU, TBSU, ASSF, LANCE <i>Ultra</i> |
| ERK1/2 total | ALSU, MPSU, LANCE <i>Ultra</i> , LANCE |
| ERK1/2 phospho Y204 | ALSU, LANCE <i>Ultra</i> , LANCE |
| MEK1 total | HTRF, ALSU, Alpha CETSA |
| MEK1 phospho S218/222 | HTRF, ALSU, TBSU, ASSF |
| MEK1 phospho S298 | HTRF |
| MEK1 phospho S218/222 and total | MPSU |
| MEK1/2 phospho S217/221 | HTRF, LANCE <i>Ultra</i> |
| MEK1/2 phospho S218/222 | HTRF |

| TAG-LITE LABELED RECEPTORS AND LIGANDS | GPCR |
|--|----------------------------|
| Adenosine A1 | Adenosine A1 receptor |
| Adenosine A2A | Adenosine A2A receptor |
| Adenosine A2B | Adenosine A2B receptor |
| Adenosine A3 | Adenosine A3 receptor |
| Adrenergic beta-1 | Adrenergic beta-1 receptor |
| Adrenergic beta-2 | Adrenergic beta-2 receptor |
| Angiotensin AT2 | Angiotensin AT2 receptor |
| Chemokine CXCR4 | Chemokine CXCR4 receptor |
| Dopamine D2 | Dopamine D2 receptor |
| Glucagon GIPR | Glucagon GIPR receptor |
| Glucagon GLP1 | Glucagon GLP1 receptor |
| Orexin OX2 | Orexin OX2 receptor |
| Serotonin 5HT1A | Serotonin 5HT1A receptor |
| Serotonin 5HT4 | Serotonin 5HT4 receptor |
| Vasopressin V2 | Vasopressin V2 receptor |
| Delta-opioid | Delta-opioid receptor |
| Kappa-opioid | Kappa-opioid receptor |
| Mu-opioid | Mu-opioid receptor |

| GTP BINDING ASSAY KITS | TECHNOLOGY |
|--------------------------|------------|
| GTP / Gi protein binding | HTRF |

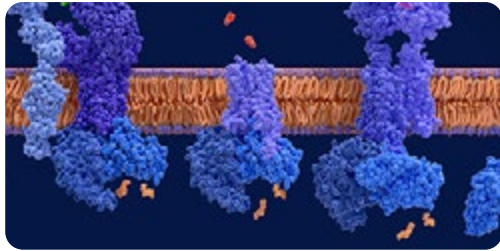
| SECOND MESSENGER ASSAYS | TECHNOLOGY |
|-------------------------|--|
| cAMP | AlphaScreen, HTRF, AlphaLISA, LANCE <i>Ultra</i> , LANCE |
| IP-One | HTRF, AlphaLISA |

| β-ARRESTIN ASSAY KITS | TECHNOLOGY |
|--------------------------|------------|
| β-arrestin 2 recruitment | HTRF |
| β-arrestin 1 total | HTRF |
| β-arrestin 2 total | HTRF |
| AP2 total | HTRF |

ALSU = AlphaLISA *SureFire Ultra*; ASSF = AlphaScreen *SureFire*; MPSU = Alpha *SureFire Ultra* Multiplex; TBSU = Terbium *SureFire Ultra*

GPCR Cell Lines and Membrane Preparations

With more than 400 stable cell lines for binding and functional testing, over 150 frozen ready-to-use cells, plus more than 100 membrane preparations, ours is one of the largest portfolios of validated GPCR cell lines in the business.

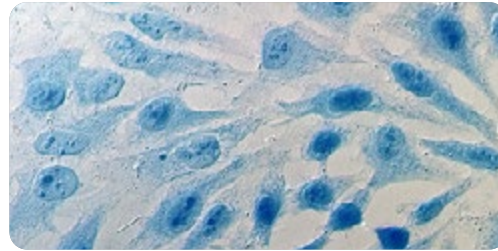


Membrane Preparations

Identify and characterize ligands that bind to your receptor of interest with our membrane target systems, prepared from cells that express recombinant or endogenous GPCRs.

Targets include:

- Adenosine
- Adrenergic
- Cannabinoid
- Dopamine
- GABA
- Melanocortin
- Muscarinic
- Opioid
- Serotonin



Stable Cell Lines

Decrease assay development time and increase the quality of your results with our validated GPCR stable cell lines for binding and functional testing and for calcium analysis.

Targets include:

- Adenosines
- Adrenoceptors
- Cannabinoids
- Chemokines
- Dopamine
- Galanin
- Histamines
- Opioids
- Muscarinic



Frozen Cells

Perform cellular GPCR tests on multiple receptors at a time with frozen, validated, ready-to-use cells for streamlined screening, lead optimization, and profiling.

Targets include:

- Adrenergic
- Anaphylatoxin
- Chemokine
- Histamine
- Melanocortin
- Muscarinic

Multimode Plate Readers

Our [multimode microplate readers](#) offer all major detections such as absorbance, Alpha, luminescence, fluorescence intensity, fluorescence polarization, and time-resolved fluorescence, all in one instrument. Our EnSight® multimode plate reader also offers fast well-imaging, so you can perform target-based and phenotypic screening to gain valuable insights from different perspectives. All our plate readers are configurable, so you can add technologies when you need to futureproof your lab. With over 30 years' experience in instrument development, you can expect performance and reliability from all our microplate readers.



eBook: Choosing the Right Plate Reader



Infographic: Accelerating Drug Discovery

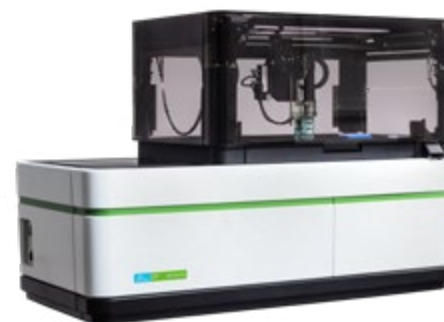


EnSight, VICTOR® Nivo™, and EnVision® Multimode Plate Readers

High-Content Imaging

Our high-content imaging devices enable you to interrogate the modification of GPCR homeostasis signaling at the single-cell or well levels, in brightfield and fluorescence with 2D and 3D cell models. The flagship [Opera Phenix® Plus high-content imager](#) is equipped with onboard liquid handling for fast kinetic measurement of calcium flux mobilization, including agonist and antagonist studies, while the workhorse [Operetta CLS™](#) can help you determine receptor internalization, β -arrestin localization, or other signal transduction pathways with phosphorylated or total protein expression markers.

When a phenotypic screening approach is needed, put our imaging systems together with our comprehensive portfolios of cell-line, [CRISPR reagents](#), [PhenoVue™ cell painting reagents](#), and Signals™ informatic tools. Our [MuviCyte live-cell imager](#) is perfect for longitudinal kinetic imaging applications. And our Cellometer and Cellaca devices are ideal for your all-important of cell counting applications.



Opera Phenix Plus High-Content Screening System



Operetta CLS High-Content Analysis System



Application Note: Kinetic Calcium Flux Imaging with Opera Phenix Plus



Application Note: Phenotypic Analysis of Hypertrophy in Human iPSC-Derived Cardiomyocytes



Application Note: ERK Signal Transduction in Live Cells Using FRET/Operetta CLS HCA



Application Note: Cell Painting for Phenotypic Screening

Solutions for Your Drug Discovery Workflow

Advancing disease research and bringing new drugs to market quickly is supported by innovative testing and analysis solutions that deliver fast, accurate, and reproducible results, leading to novel insights. Our robust portfolio of solutions helps you meet key criteria across critical lab workflows.

Automated Liquid Handling

With dedicated workstations designed for a diverse variety of applications, along with the ability to customize liquid handling based on your needs, our innovative solutions enable you to minimize errors, reduce hands-on time, and increase throughput and reproducibility. And with flexibility in throughput, capacity, and dynamic volume range; high-quality manufacturing standards; and outstanding customer service and support, they can meet the needs of even the highest volume labs.

Automated Cell Counting for GPCR Screening

With our high-throughput solutions, you can measure live-cell concentration, viability, and cell size distribution in a single cell sample, or in multiple cell samples simultaneously. You can perform traditional trypan-based or advanced fluorescence-based viability assays for GPCR-expressing cell lines for accessible cell sample data. What's more, you can quickly and automatically capture and document cell images and size histogram reports to monitor cell-line quality for efficient and reliable workflows.



Whole-Genome Screening with CRISPR

CRISPR genome editing provides a highly efficient way to find drug targets and probe gene function by generating gene knockouts. Our optimized screening platform, sophisticated bioinformatics analysis, and ready-made custom libraries allow for the design of the most appropriate workflow for your screening project, providing exceptional data quality. We can take care of the entire workflow or work with you to execute the project in your own lab.



Microplates for Research

Our OptiPlates™, AlphaPlate™, and ProxiPlates™ research-grade microplates, available in black, gray, and white, are designed to give you optimal performance. They're made using quality plastic injected into a mold in a clean room. Together with our strict QC tolerance testing, this ensures that you're getting the best-quality microplate possible. In addition, we offer a selection of treatments and coatings that fit all your research needs.

Microplates for Cell Imaging

Drawing on many years of experience, we've developed PhenoPlate™ microplates as a key part of our complete solution for high-content applications. These microplates have been engineered to deliver superior images and the highest quality data for high-content applications. We also offer ViewPlate™ microplates engineered for bottom-reading systems and high-content imaging, which can be used for a range of cell-based assay applications, including imaging, fluorescence, luminescence, and absorbance, as well as many cell-culture applications.

Image Analysis and Management

Our Signals Image Artist™ image data storage and analysis system is an instrument-independent image analysis and management platform. It's the only system that provides universal high-volume image-data storage and analysis and brings access to images from a wide range of sources, including all major high-content screening instruments.

For more information visit www.perkinelmer.com/category/gpcr-research

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