bio:ascent

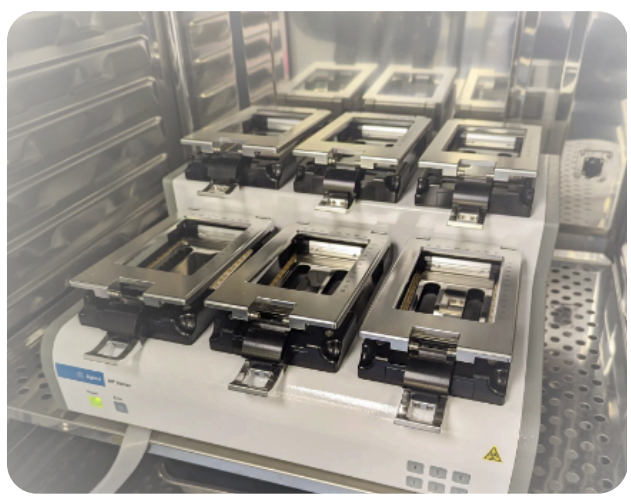
# GPCR ASSAY TECHNOLOGIES GUIDE

# CELLULAR IMPEDANCE

bio:ascent

## HOW IT WORKS

Microelectronic biosensor system that monitors whole-cell responses without labels



## TECHNOLOGY

Agilent xCELLigence cell analysis system

## KEY FEATURES

**Label-free** functional assay

**Broad applicability** across various cell types: endogenous GPCRs in primary cells, over-expressing cell lines, stem cells, or disease relevant cell lines

Simultaneously screen GPCR function across **all coupling classes**: Gαs, Gαq, as well as Gαi and Gα12/13

## INSIGHTS GENERATED

**Real-time, whole-cell kinetic data** enables deep, mechanistic insight into GPCR signalling

Interrogation of **complex signalling pathways** with high resolution, especially useful for recptors with **unknown signalling profiles** (eg. Orphan receptors)

Gain a **differentiated** view of GPCR pharmacology that helps uncover **nuanced signalling signatures and identify functional selectivity**

## YOUR PARTNER FOR SUCCESS IN GPCR DRUG DISCOVERY

# CELLULAR IMPEDANCE

bio:ascent

## CASE STUDY

5-$HT_{2A}$ is a GPCR which is abundantly expressed in the CNS and an attractive clinical target for anxiety, psychosis and OCD.

MDL 100907 is characterised in the literature as a selective 5-$HT_{2A}$ antagonist. Profiling of MDL 100907 in the xCELLigence assay revealed that this ligand also displays inverse agonist activity at the 5-$HT_{2A}$ receptor. This result highlights the importance of incorporating orthogonal assay approaches into pre-clinical drug safety and efficacy profiling.

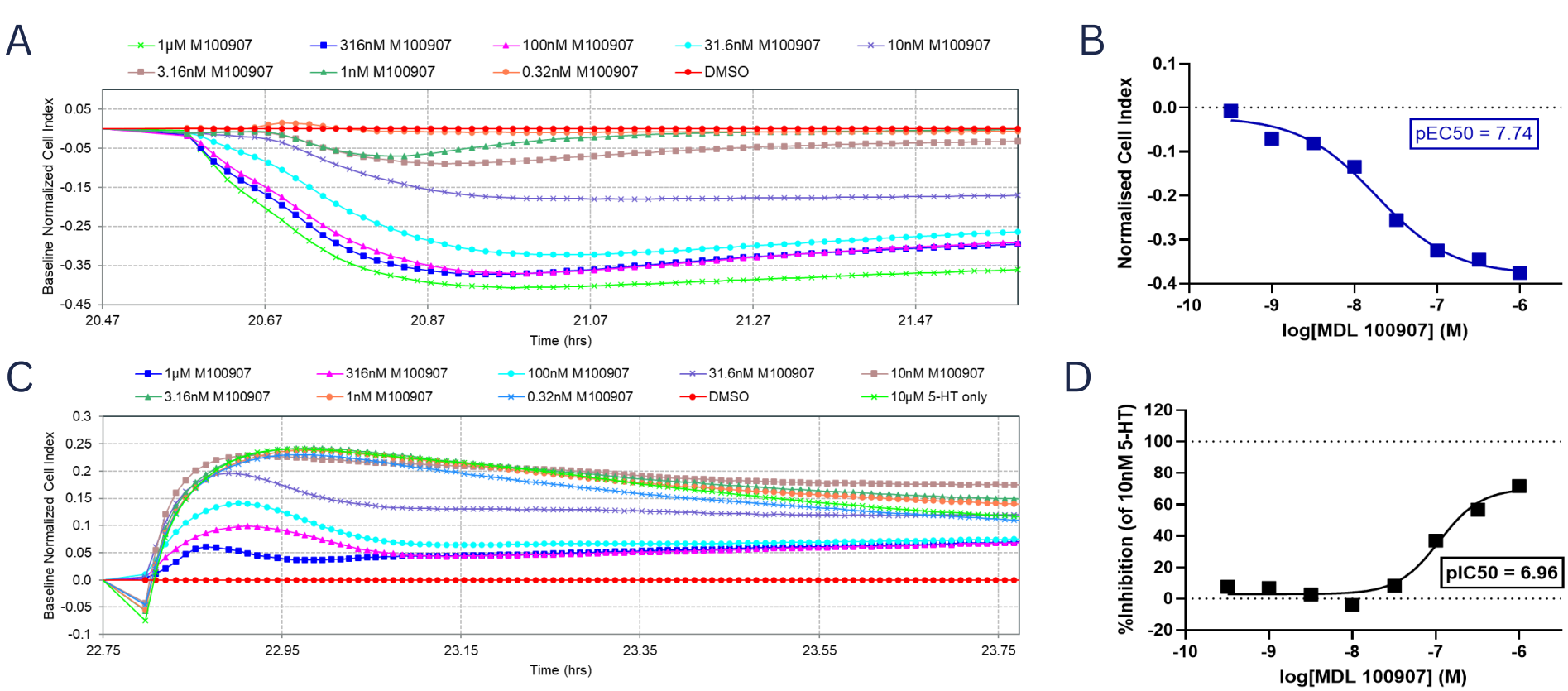



**Figure 1.** MDL 100907 shows antagonist and inverse agonist activity at the 5-$HT_{2A}$ receptor in the label-free xCELLigence assay. Baseline normalised cell index trace for MDL 100907 alone (A) or 10nM 5-HT ($EC_{80}$) following pre-treatment with the indicated concentrations of MDL 100907 (C). Normalised cell index values plotted as a dose-response curve for MDL 100907 alone (B) or dose-inhibition curve for MDL 100907 pre-treatment, plotted at the peak agonist response (D).

# β-ARRESTIN RECRUITMENT bio:ascent

## HOW IT WORKS

A live cell, luminescence based protein-protein interaction assay measuring β-arrestin recruitment to GPCRs in real time

## TECHNOLOGY

Promega NanoBiT Arrestin

## KEY FEATURES

Generic method for all GPCRs; **useful for non G protein pathways**

Useful for **biased agonsim** studies

Useful for orphan receptors where endogenous ligands/ **coupling pathways are unknown**

Readily **scaled** for HTS

## INSIGHTS GENERATED

Uncovers GPCR pharmacology **beyond traditional G-protein signalling**

Quantifies **ligand bias**. Helps determine whether compounds favour G-protein signalling, β-arrestin recruitment, or demonstrate a balanced profile

Supports the development of **safer, more targeted therapeutics** by enabling **precise control** over downstream signalling pathways

## YOUR PARTNER FOR SUCCESS IN GPCR DRUG DISCOVERY

# CALCIUM FLUX

bio:ascent

## HOW IT WORKS

Fluorescent dye and high speed plate imaging system detects intracellular $Ca^{2+}$ changes


## TECHNOLOGY

FLIPR Penta

## KEY FEATURES

Pathway specific for **Gαq signalling**

Can be used with **Gα16-engineered** systems, enabling receptors to be “forced” through the calcium pathway for rapid functional assessment

Highly **sensitive** to early signalling

FLIPR Penta - extremely **high-throughput**

## INSIGHTS GENERATED

Captures **full real-time** fluorescence traces, going far **beyond simple max–min endpoints**

Detailed **interrogation of signalling behaviour**, including onset time, peak amplitude, and the rate of signal decay as cells return to baseline

**Rich kinetic insight** supports deeper mechanistic understanding, ligand differentiation, and confident decision-making

## YOUR PARTNER FOR SUCCESS IN GPCR DRUG DISCOVERY

# IP1 ACCUMULATION

bio:ascent

## HOW IT WORKS

Measures accumulation of IP1 caused by Gαq activation using TR-FRET

## TECHNOLOGY

HTRF IP-One assay

## KEY FEATURES

Suitable for **Gαq-coupled GPCRs**

Functional assay for live cells which works with **many cell systems**

Robust assay suitable for **miniaturisation and HTS** cascades

## INSIGHTS GENERATED

Directly **quantifies Gαq-coupled** GPCR signalling

Unlike rapid and transient calcium flux, $IP_1$ accumulation provides a **longer, more forgiving measurement window**, delivering a stable and **highly reproducible** readout

Ideally suited for HTS applications - this assay is an **excellent choice for reliable compound screening**

## YOUR PARTNER FOR SUCCESS IN GPCR DRUG DISCOVERY

# CAMP MEASUREMENT

bio:ascent

## HOW IT WORKS

Measures intracellular cAMP levels as a readout of Gαs or Gαi/o signalling

## TECHNOLOGY

Promega Glosensor cAMP
Bellbrooks transcreener cAMP
Revvity HTRF cAMP

## KEY FEATURES

Highly **sensitive, quantitative** readout

Versatile - suitable for **both Gαs and Gαi** receptors

**High-throughput** format ideally suited to HTS

**Flexible** - performs equally well in endogenously expressing cell lines as in stably or transiently transfected systems

## INSIGHTS GENERATED

**Reliable insight** into compound behaviour and receptor signalling - kinetic insight empowers deeper mechanistic understanding and more confident decision-making

Excellent for **SAR generation and medicinal chemistry** due to quantitative data

High sensitivity enables detection of **weak agonists, partial agonists, and inverse agonists**

## YOUR PARTNER FOR SUCCESS IN GPCR DRUG DISCOVERY

# CAMP MEASUREMENT

bio:ascent

## CASE STUDY

During a long running Lead Optimisation project on a GPCR target, we observed some very steep SAR, which was unexpected.

The possibility of silent allosteric modulators (SAMs) was investigated using the functional cAMP primary assay. We pre-incubated ± the suspected SAM with a known allosteric antagonist before the addition of the agonist.

Dis-inhibition of the antagonist with no agonist activity on its own indicates that the compound is indeed binding at the same site but doing so without causing a functional effect.

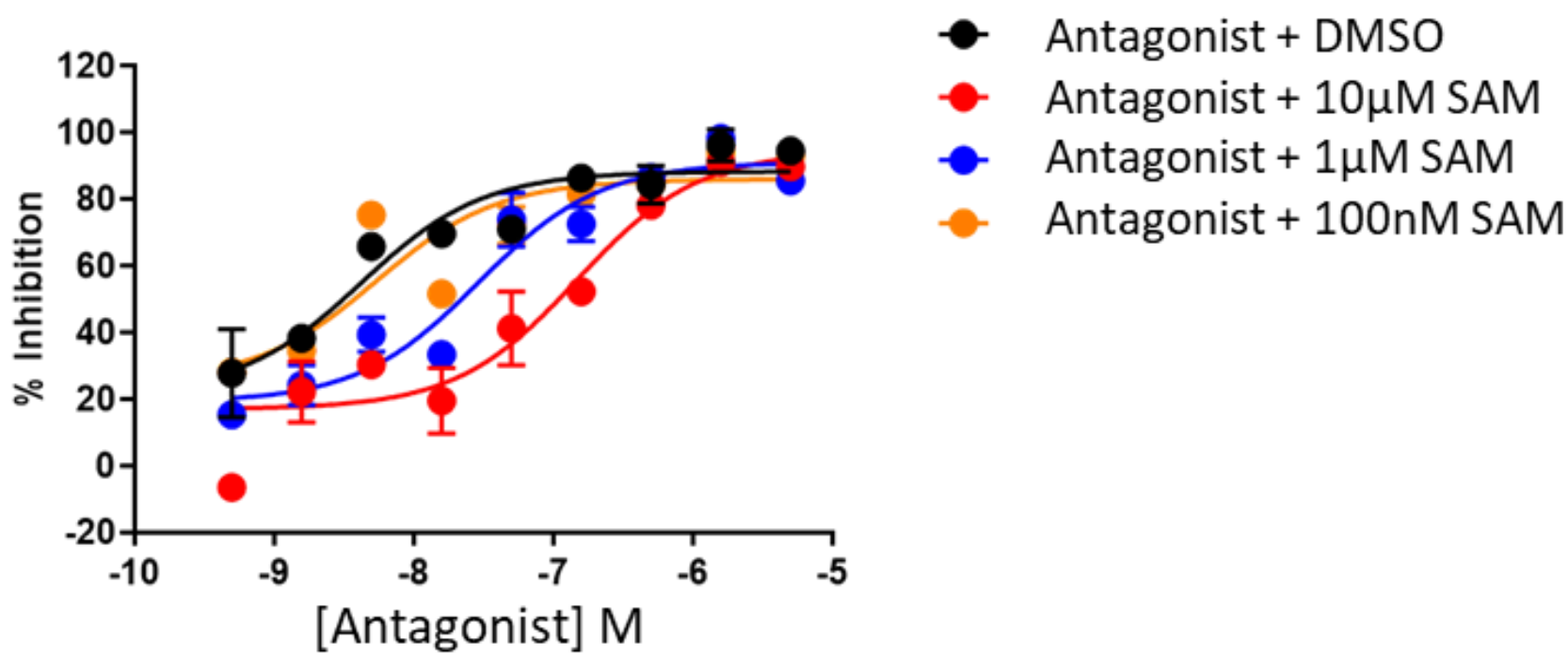



**Figure 2.** Allosteric antagonist dose response curve ± increasing concentrations of potential silent allosteric modulator in the presence of agonist $EC_{80}$. Data are expressed as percent inhibition of the agonist $EC_{80}$ response and are the mean ± SEM of a minimum of 4 replicates.

# PHOSPHO-ERK

bio:ascent

## HOW IT WORKS

Measures phosphorylation of ERK1/2, a key downstream signaling output of many GPCRs

## TECHNOLOGY

Alphascreen Phospho-ERK assay

## KEY FEATURES

**Universal** readout - suitable for Gαq-, Gαi/o-, and some Gαs-coupled receptors

Sensitive technique which can detect **weak or partial agonists**

Captures **both** G-protein and β-arrestin mechanisms

## INSIGHTS GENERATED

Provides a readout of integrated GPCR signalling - especially valuable for receptors that are **difficult to monitor using traditional second-messenger assays**

Captures ERK as a convergence point for both G-protein dependent and β-arrestin mediated pathways, offering a **broad, biologically meaningful view** of receptor activity

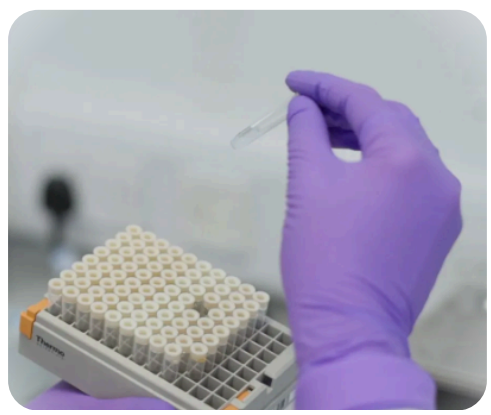
Enables **reliable detection** of ligand-driven signalling events

**YOUR PARTNER FOR SUCCESS IN GPCR DRUG DISCOVERY**

# TRUPATH

bio:ascent

## HOW IT WORKS



Directly monitors G-protein activation by tracking the separation of tagged Gα and Gγ subunits

## TECHNOLOGY

Bioluminescence Resonance Energy Transfer (BRET)

## KEY FEATURES

Reports proximal, immediate G-protein engagement, letting you see exactly **which G-protein family a compound activates**

Delivers a **depth of mechanistic pharmacology** that traditional assay formats simply cannot achieve

**Optimised** constructs available for nearly every Gα, Gβ, and Gγ subtype

## INSIGHTS GENERATED

Enables **precise mapping** of GPCR G-protein coupling and signaling bias - reveals exactly which G-proteins a receptor couples to, and the strength of those interactions

Enables the discovery and optimisation of ligands with **finely tuned, G-protein–specific bias**

Uncovers **unique signalling signatures** - particularly valuable when working with first-in-class compounds or orphan receptor programmes

## YOUR PARTNER FOR SUCCESS IN GPCR DRUG DISCOVERY

bio:ascent

# READY TO MOVE YOUR GPCR PROJECT FORWARD?

INFO@BIOASCENT.COM