HUMAN HEALTH

ENVIRONMENTAL HEALTH

MAKE AN EPIC DIFFERENCE IN YOUR RESEARCH

LABEL-FREE TECHNOLOGY

Label-free Solutions

PerkinElmer®
For the Better

ARRIVE AT YOUR BIG DECISIONS SOONER

Now you can characterize cellular signaling mechanisms and better understand the complexity of multiple signaling pathways more efficiently. Only the EnSpire® Multimode Plate Reader offers established Corning® Epic® label-free technology in addition to labeled technologies all within one flexible benchtop reader.

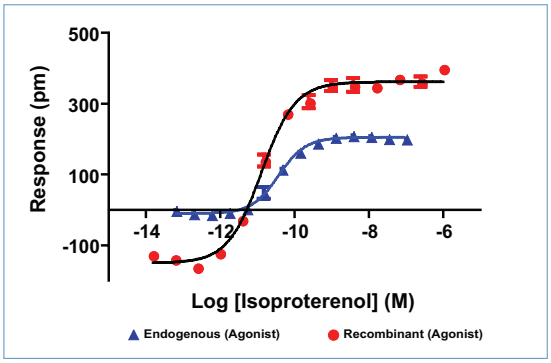
This exclusive cellular and biochemical label-free platform, in conjunction with labeled technologies, provides a unique orthogonal approach, enabling you to make better decisions faster.

The EnSpire label-free platform offers:

- More fully characterized information about both cellular and biochemical systems
- Pathway-independent analysis
- Non-invasive, more physiologically relevant data
- Ability to study difficult targets or weak biological interactions

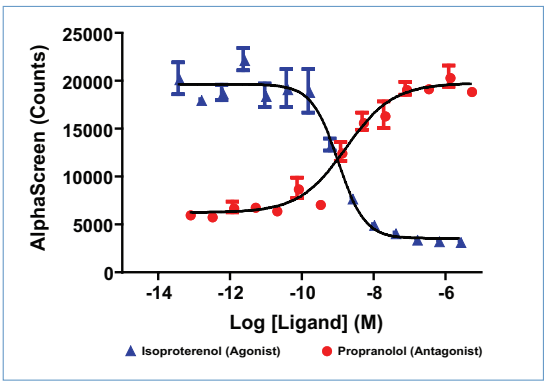
Pathway-unbiased, label-free technology offers rich information for difficult targets, endogenously expressed receptors and recombinant cell lines in a 384- or 96-well plate format.

Pharmacology Shows Robust Detection of Endogenous and Recombinant Targets



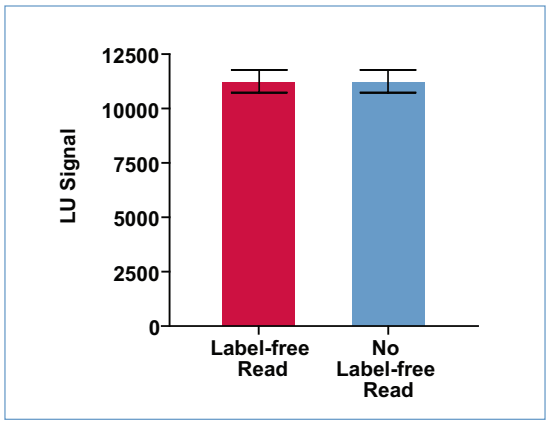
Comparable agonist dose response curves using recombinantly β_2 (CHO-K1) and endogenously (A431) expressed β_2 GPCR targets can be obtained from different host cells and either freshly passaged or frozen irradiated cells in a label-free 384-well assay (18,000 cells/well).

Orthogonal Assay Results on Label-free Platform



β_2 agonist and antagonist dose response curves using AlphaScreen® cAMP confirm the β_2 pharmacology from the EnSpire label-free platform (384-well, 10,000 cells/well). See page 3 for pharmacology data.

Cell Viability Unaffected by Label-free Scans

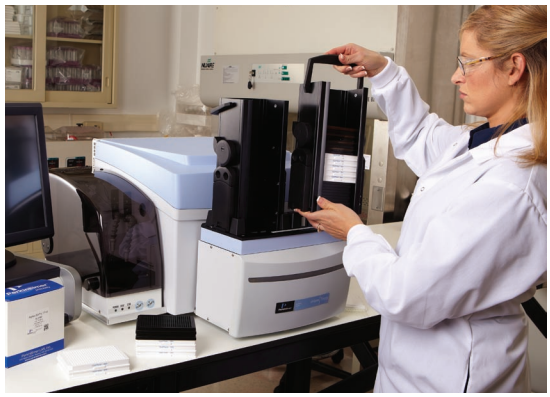


Sequential assays can be performed following a label-free assay as demonstrated with ATPLite® luminescence which measures cell viability.

Unparalleled versatility: the EnSpire label-free platform

Orthogonal research using labeled and label-free assays has never been easier. Choose the Epic® label-free platform and patented Alpha Technology, ultra-sensitive luminescence, fluorescence intensity or absorbance for a truly versatile detection system – all in a convenient benchtop reader. The EnSpire label-free platform includes specially designed microplates with highly precise optical sensors embedded in each well which are integral to assay performance.

Cellular label-free microplates offer cost-effective flexibility for many cell types including adherent and suspension cells and mammalian and primary cells. Biochemical label-free microplates incorporate patented dual-sensor self-referencing technology for protein:ligand assays, ensuring that only one true analyte binding is reported.



Only the EnSpire benchtop plate reader detects labeled and label-free assays.

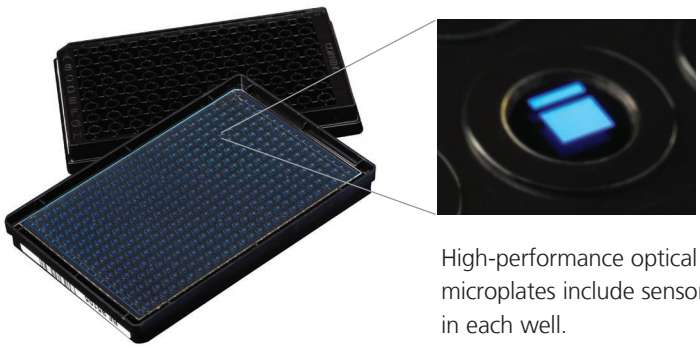
EnSpire Label-free Platform Offers Powerful Performance for GPCR Assays

			EnSpire Label-free		Epic® Label-free		EnSpire Labeled Assay**	
Target	Host Cell	Culture Conditions	Agonist EC_{50}*	Antagonist IC_{50}*	Agonist EC_{50}	Antagonist IC_{50}	Agonist EC_{50}	Antagonist IC_{50}
β_2AR (G_s)	A431 (Endogenous)	Passaged	0.03	6.2	0.02	6.0	1	1.8
β_2AR (G_s)	CHO-K1 (Recombinant)	Frozen	0.01	0.3	–	–	1	1.4
Opioid Mu (G_i)	CHO-K1 (Recombinant)	Frozen	0.9	43	0.5	35	2.3	0.03
A_1 (G_i)	HEK293 (Endogenous)	Passaged	47	35	100	153	–	–
M_1 (G_q)	HEK293 (Endogenous)	Passaged	5,900	2.2	5,000	2.0	–	–
M_1 (G_q)	CHO-K1 (Recombinant)	Passaged	177	6.3	–	–	85	14

*Potency in nM **Labeled Assays: AlphaScreen cAMP (β_2AR, Opioid Mu), Ca^{2+} Flux Assay (M_1)

Label-free pathway-independent response is demonstrated across GPCR pathways including more difficult G_i receptors. Pharmacological response was independent of whether cells were endogenous or recombinant or whether freshly passaged or frozen.

Highly Sensitive Microplates for Highly Effective Research



High-performance optical microplates include sensors in each well.

CELLULAR ASSAYS
THE POWER TO SEE THE BIGGER PICTURE

Label-free technology offers rich, physiologically relevant information with superior sensitivity from both recombinantly and endogenously expressed targets.

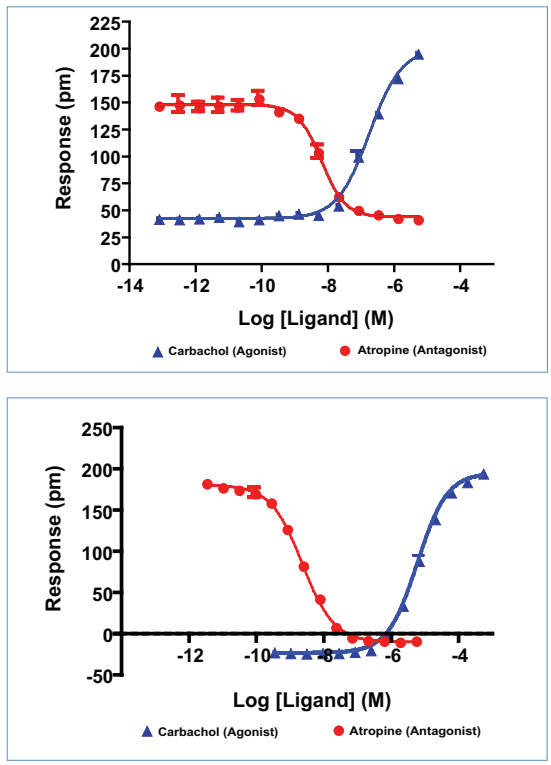
With the EnSpire label-free platform, you will see equivalent dose responses across multiple GPCR targets to that of the Epic® label-free system, and comparable pharmacology to that of labeled assays.

The integrated cellular response obtained from a label-free assay enables characterization of the signaling pathways involved that can be affected by biased agonism, dimerization and allosterism.

Typical cellular label-free assays include:

- GPCRs and orphan receptor screening
- Pathway identification and validation
- Receptor panning
- Receptor tyrosine kinases
- Ion channels

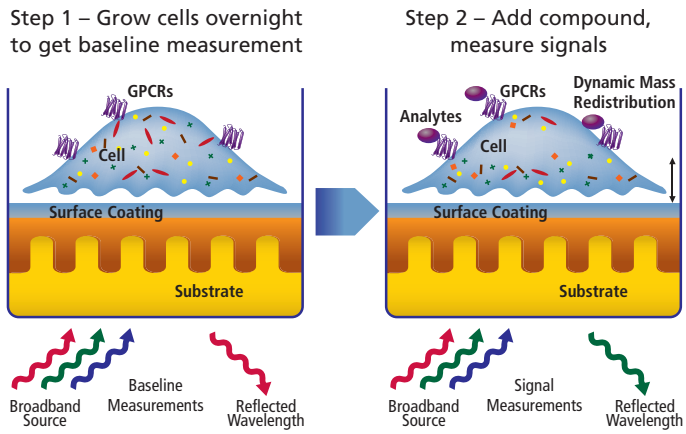
Label-free Detection Precisely Differentiates GPCR Signaling



Shown is agonist and antagonist (G_q) response to recombinant receptor M_1 in CHO-K1 cells (top) and endogenous HEK293 cells (bottom).

Cellular assays

Epic® technology measures changes in light refraction resulting from dynamic mass redistribution (DMR) within the cell which occurs in response to receptor activation or deactivation in a zone within the cell's monolayer. The change is indicated by a change in wavelength. There is an integrated response for every pathway, which enables the detection of cellular responses for endogenous as well as recombinantly expressed targets with great sensitivity.



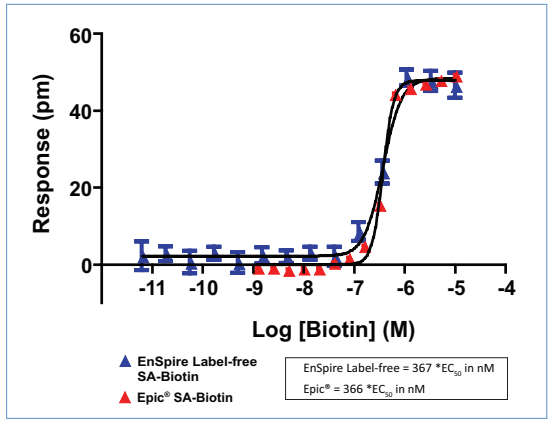
BIOCHEMICAL ASSAYS
AN EASIER WAY
TO STUDY DIFFICULT TARGETS

Perform highly sensitive, label-free biochemical binding assays with confidence, including difficult targets or weak biological interactions. You can look forward to the same high performance with the EnSpire label-free platform that has been demonstrated with the established Corning® Epic® System.

Typical biochemical label-free assays include:

- Screening binding strength (K_D) assays that complement SPR technology
- Detection of direct biomolecular interactions
- Proteases
- Protein-oligo (DNA/RNA) interactions

EnSpire vs. Corning® Epic® System (protein:protein interaction assay)



Excellent comparability of the two label-free platforms is demonstrated in this streptavidin-biotin dose response curve.

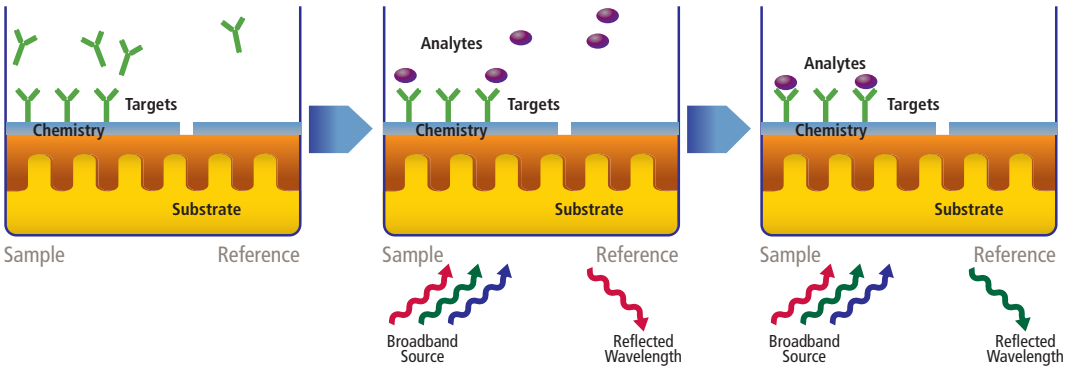
Biochemical assays

Label-free biochemical assays measure changes in the index of refraction upon a binding event. As in cellular assays, the change is indicated by a shift in wavelength.

Step 1 – Target is immobilized on the microplate amine-coupling surface

Step 2 – Reference area prevents non-specific target immobilization. Wash and baseline read performed. Then analyte added

Step 3 – Analyte is bound, allowing for the final read



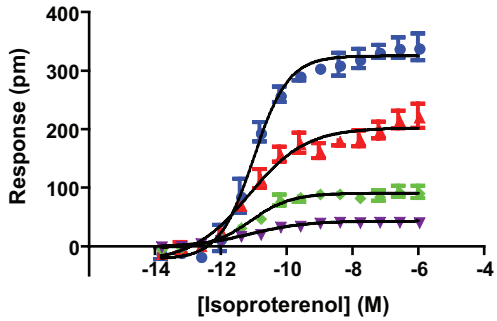
ESSENTIALS FOR A COMPLETE LABEL-FREE SOLUTION

For greatest convenience, choose from a wide portfolio of GPCR cell lines and frozen cells validated using several biochemical and functional assays including label-free technology. Label-free also enables you to work with subconfluent cells and more difficult GPCR targets which may not provide adequate signal-to-background using a conventional assay.

Automated liquid handling: for the highest quality results

As a complement to your EnSpire Multimode Plate Reader, automated liquid handling ensures reproducible and consistent results throughout your assay development process. JANUS® Automated Workstations can support your assay development needs via a standalone instrument or integrated to other devices to achieve your desired level of "walkaway" automation. With predefined and validated templates for your label-free assays, any user can perform the required protocol/setup with greater ease and confidence.

Cell Titration with Recombinant β_2AR and Agonist in CHO-K1 Cells



Cell Density	EC_{50} (pm)
16,000	10
8,000	7
4,000	8
2,000	7

Cells were seeded 24 hours prior to the assay in 384-well label-free microplates. This cell titration shows the ability to work with subconfluent cells, suggesting future applicability for primary and stem cells.

The EnSpire stacker module includes 20- or 50-plate magazines, and is compatible with the PlateStak® Automated Microplate Handler to allow efficient manual transfer of plates to the EnSpire.

Make an epic difference in your drug discovery research from hit-to-lead to target confirmation and lead optimization. Only EnSpire offers fully orthogonal research – labeled and label-free detection – all on one flexible platform. For more information visit **www.perkinelmer.com/EnSpireLabelfree**.

The incorporation of label-free Epic® technology into this client's drug discovery process has improved process both in assay development and post-HTS screening for structure-activity-relationship (SAR) and hit-profiling studies.

Assay Type

Fluorescence Ca^{2+} flux assay using a FLIPR$^{TETRA®}$ High Throughput Cellular Screening System and confirmation studies using the Corning® Epic® label-free system.

Challenge

There was a need for highly sensitive counter-screening across different targets within hit follow-up to ensure potential lead compounds were more fully characterized prior to decisions regarding their progress.

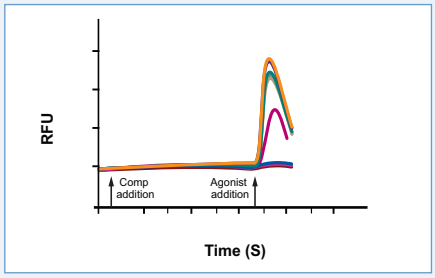
Results

Novel compound activities that are missed by a traditional labeled method can be revealed using Epic® technology. In the top figure, a compound was tested in a FLIPR® Ca^{2+} flux assay and shown to behave as a neutral antagonist. The Epic® DMR response shown in the middle figure similarly demonstrates the antagonist activity of the compound but additionally identifies inverse agonist activity after the first addition. No such response was observed in the FLIPR® Ca^{2+} flux assay.
The dose response data for antagonist activity (yellow curve) and inverse agonist activity (blue curve) on Epic® is shown in the bottom figure.
For assay development, the process benefited from being both more rapid and generic.

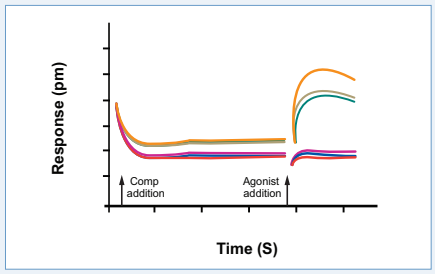
Conclusions

Label-free technology is ideal for use in drug discovery, enabling the study of GPCRs and cell signaling. It has already been adopted within the hit-to-lead process for target confirmation and lead optimization within secondary and orthogonal screening, SAR studies, biophysical testing and ADME/Toxicity.

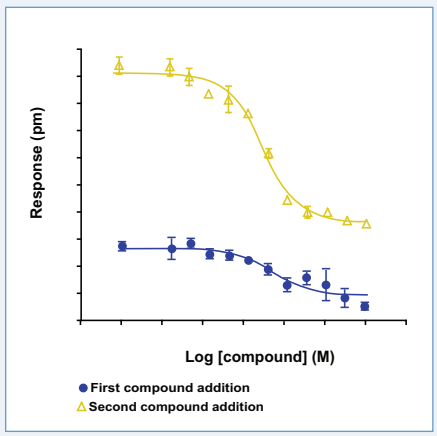
FLIPR® Response Identifying 1st and 2nd Compound Additions



Epic® Response Identifying 1st and 2nd Compound Additions



Epic® Dose Response Curves Identifying 1st and 2nd Compound Additions



For scientific breakthrough.
For a better tomorrow.

At PerkinElmer, we share your commitment to finding answers to the mysteries of human health. With proven expertise in reagents, assays, cellular imaging, detection systems and automated liquid handling, we offer the right combination of technologies and service, enabling scientists around the world to rapidly discover new therapies.

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