# Gain confidence in your lead compound

## Products and services for drug metabolism and safety

invitrogen™



# Invitrogen products and services for drug metabolism and safety

→ Obtain key information on metabolic stability

→ Uncover potential drug–drug interactions with drug-metabolizing enzymes

→ Determine safety profiles of lead candidates

Obtaining information about the pharmacological, toxicological, metabolic, and kinetic properties of new drug candidates is critical during the early stages of drug discovery and development. Invitrogen offers a collection of validated tools including P450 microsomes and a variety of assay tools and services to help you acquire the necessary information about metabolic stability, interactions with drug-metabolizing enzymes, and safety profiles of lead candidates.

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# Drug-metabolizing enzymes and assays

Drug-metabolizing enzymes (DMEs) are a diverse group of enzymes located primarily in the liver. They are responsible for metabolizing a vast array of xenobiotic compounds including drugs, environmental pollutants, and endogenous compounds such as steroids and prostaglandins. There is an increasing effort to integrate metabolism studies early in the drug discovery process because poor pharmacokinetics account for a substantial fraction of clinical failures. In addition, high-profile withdrawals of drugs from the market have highlighted the importance of understanding drug–drug interactions. To accelerate these efforts, Invitrogen provides recombinant drug-metabolizing enzymes and assay methods that enable scientists in the pharmaceutical setting to screen large numbers of diverse compounds for metabolism and P450 inhibition profiles.

## P450 isozymes well suited for high-throughput screening

Cytochrome P450 BACULOSOMES® reagents offer a distinct advantage over human liver microsomes (HLMs) in that only one P450 enzyme is expressed, preventing metabolism by other P450s (or other classes of DMEs) and allowing analysis of a single isozyme. Cytochrome P450 BACULOSOMES® reagents are microsomes prepared from insect cells infected with a recombinant baculovirus expressing a specific human P450 isozyme and a rabbit NADPH-P450 reductase.

Compound rankings based on metabolism and inhibition profiles observed with the P450 BACULOSOMES® reagents are very similar to those seen with HLMs for most compounds tested. The activity and metabolic rate of the P450 BACULOSOMES® reagents with most substrates, however, are significantly higher than those observed with preparations of HLMs. This results in a broad dynamic range and high sensitivity in assays utilizing P450 BACULSOMES® reagents. The high levels of activity and reproducibility of the enzyme component of the reaction with most probe substrates make the P450 BACULOSOMES® reagents well suited to high-throughput screening formats. In fact, P450 BACULOSOMES®–based assays have been miniaturized to a 2 µl format for use with Vivid® fluorescent substrates. Data generated using the P450 BACULOSOMES® reagents in conjunction with the Vivid® high-throughput assay platform correlate well with LC-UV data, as demonstrated by the CYP3A4 isozyme (Figure 1).

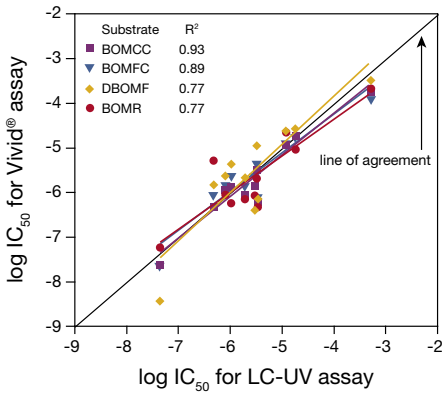



**Figure 1—CYP3A4 Vivid® assays with BACULOSOMES® reagents correlate well with LC-UV assays.** $IC_{50}$ values for 12 known CYP3A4 inhibitors were obtained using four distinct Vivid® substrates, and compared to values generated using a traditional testosterone 6 beta-hydroxylation LC-UV assay with CYP3A4 BACULOSOMES® reagent. The Vivid® assays were highly predictive of LC-UV assay results.

To learn more about P450 BACULOSOMES® reagents, visit www.invitrogen.com/baculosome.

## Speed your selection of novel lead compounds

Vivid® CYP450 Screening Kits are designed to rapidly screen compounds early in the drug discovery process for P450–drug interactions and to generate data for predictive structure–activity relationship (SAR) modeling. Vivid® CYP450 Screening Kits provide the high performance, throughput, and reliability you need to speed selection of novel compounds for drug development. These assays are:

- → Easy and homogeneous—three-step mix-and-read format, no stop reagents required
- → Rapid and flexible—kinetic or endpoint mode, at room temperature or 37°C
- → Robust—high signal-to-background ratios and excellent Z´-factors
- → Miniaturizable—assays can be run in 96-, 384-, or 1,536-well plates

Vivid® Substrates are blocked dyes that yield minimal fluorescence until oxidative cleavage occurs (Figure 2A). Metabolism at either of two potential cleavage sites releases the highly fluorescent product. P450 inhibitors are identified by their ability to prevent the production of the fluorescent metabolite. The three-step Vivid® screening assay (Figure 2B) is a simple mix-and-read protocol that does not require stop reagents to conduct the reaction in kinetic mode.

Vivid® kits and substrates are available for a variety of P450 isozymes (Table 1). Each kit includes the isozyme-specific P450 BACULOSOMES® reagent, a Vivid® substrate, and an NADPH regeneration system. The excitation and emission wavelengths of cleaved Vivid® Substrates are in the visible region and are subject to little or no fluorescent interference from NADPH or most test compounds.

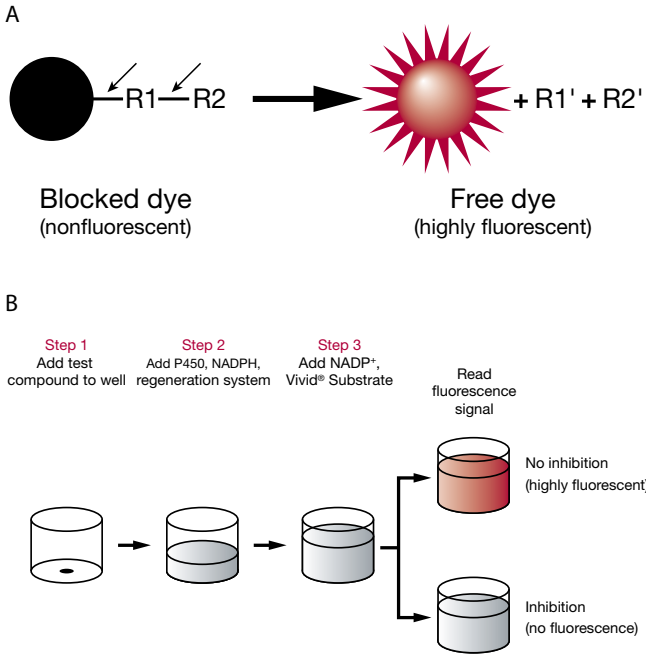
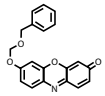
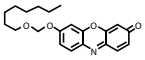
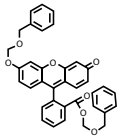
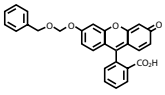
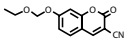
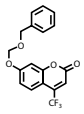
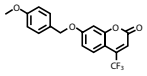



Figure 2—The Vivid® screening assay. **A.** The Vivid® substrate fluoresces when it is cleaved via P450 enzyme activity. **B.** A simple three-step protocol is used to test compounds for their ability to inhibit P450 activity. The test compound is added to a reaction containing P450 and the Vivid® substrate, and the resulting fluorescence reflects the degree of inhibition of P450 activity.

For more information on Vivid® kits and substrates, please visit www.invitrogen.com/vivid.

Table 1—Choose the right Vivid® substrate for your needs.

| Structure | Substrate | MW | Isozyme compatibility | Fluorescence color | Ex/Em* (nm) |
|---|---|---|---|---|---|
|  | Vivid® BOMR Substrate | 333.3 | CYP3A4 | Red | 530/585 |
|  | Vivid® OOMR Substrate | 355.4 | CYP2C9 | Red | 530/585 |
|  | Vivid® DBOMF Substrate | 572.6 | CYP3A4<br>CYP3A5 | Green | 485/530 |
|  | Vivid® BOMF Substrate | 452.5 | CYP2C9 | Green | 485/530 |
|  | Vivid® BOMCC Substrate | 307.3 | CYP3A4<br>CYP3A5<br>CYP2C9<br>CYP2B6 | Blue | 409/460 |
|  | Vivid® EOMCC Substrate | 245.2 | CYP2D6<br>CYP1A2<br>CYP2C19<br>CYP2E1 | Blue | 409/460 |
|  | Vivid® BOMFC Substrate | 350.3 | CYP3A4<br>CYP3A5<br>CYP2B6 | Cyan | 400/502 |
|  | Vivid® MOBFC Substrate | 350.3 | CYP2D6 | Cyan | 400/502 |

* Approximate fluorescence excitation and emission maxima.

Visit www.invitrogen.com/drugmetabolism for more information about these products.

## Access our highly active UDP-glycosyltransferase (UGT) enzymes

Examination of glucuronidation reactions catalyzed by the human UDP-glycosyltransferase (UGT) family of enzymes is essential when investigating the metabolic fate of any new drug, therapeutic agent, or potentially environmentally toxic agent. UGT activity is defined by the conjugation of glucuronic acid to a wide variety of xenobiotic and endogenous substrates containing sulfhydryl, hydroxyl, aromatic amino acid, or carboxylic acid moieties. The UGTs comprise a superfamily of integral membrane proteins of the endoplasmic reticulum, and have been subdivided into two families, UGT1 and UGT2, based on the evolutionary divergence of their genes. Invitrogen's UGT BACULOSOMES® reagents (Table 2) are produced as a microsomal fraction of Sf9 insect cells infected with a baculovirus strain containing a cDNA for a specific human UGT.

To learn more about our family of UDP-glycosyltransferase (UGT) enzymes, please visit www.invitrogen.com/UGT.

Table 2—UGT BACULOSOMES® reagents.

| Specifications | |
|---|---|
| Source | Recombinant baculovirus-infected Sf9 cells |
| Concentration | 2–20 mg/ml |
| **BACULOSOMES® reagent** | **Substrate and specific activity** |
| UGT1A1 | Octyl gallate: ≥500 pmol/min/mg |
| UGT1A6 | Naphthol: ≥500 pmol/min/mg |
| UGT1A7 | Octyl gallate: ≥150 pmol/min/mg |
| UGT1A10 | Naphthol: ≥30 pmol/min/mg |
| UGT1A3 | Octyl gallate: ≥40 pmol/min/mg |
| UGT2B7 | Hyodeoxycholic acid: ≥67 pmol/min/mg |

## Monitor CYP1A1 induction in a live-cell readout

The GeneBLAzer® CYP1A1-*bla* LS-180 cell line allows measurement of CYP1A1 induction via a beta-lactamase reporter gene (Figure 3). The beta-lactamase (*bla*) gene is inserted within the CYP1A1 promoter and delivers a readout for CYP1A1 transactivation. Ligands such as TCDD and 3-methylcholanthrene bind the aryl hydrocarbon receptor (AhR) in the cytoplasm, leading to translocation of the AhR–ligand complex to the nucleus where it forms a heterodimer with AhR nuclear translocator (Arnt). This complex can then bind the xenobiotic response element (XRE) in the CYP1A1 promoter, enhancing CYP1A1 expression. Learn more at www.invitrogen.com/cyp1a1cells.

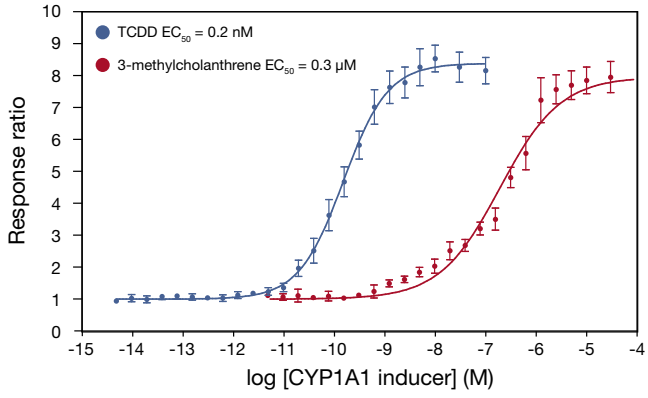



Figure 3—Dose responses of CYP1A1 inducers in the CYP1A1-*bla* LS180 assay. Cells were treated with various concentrations of 3-methylcholanthrene or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for 16 hr, then assayed for beta-lactamase activity.

# Think outside the bottleneck with our fast and reliable P450 Profiling Service

Invitrogen's P450 Profiling Service helps overcome recognized bottlenecks in drug discovery by providing timely, cost-effective determination of the inhibitory profiles of lead compounds. With this service you get:

- → Data delivered in 2 weeks
- → Strict quality control for assays and data analysis
- → Competitive pricing and confidentiality

The P450 Profiling Service utilizes Invitrogen's well-established P450 BACULOSOMES® reagents and the robust Vivid® assay platform to obtain rapid profiling of your compounds against a panel of key P450s (1A2, 2C9, 2C19, 2D6, 3A4). $IC_{50}$ determinations are performed in duplicate using 10-point titrations of test compounds.

A stringent validation process ensures the highest-quality data possible. Each data point is paired with a control well to detect interference by autofluorescent compounds. Strict quality control protocols ensure that any assay results not meeting set specifications will be automatically repeated. $IC_{50}$ value determinations for each P450 enzyme are performed with a control inhibitor on each assay plate to demonstrate validity of the data.

Figure 4 shows data for a sample inhibitor, ketoconazole, against the P450 panel. Table 3 displays rank order potency of a small panel of ligands against CYP3A4, and demonstrates excellent correlation between Vivid® and testosterone 6 beta-hydroxylation assays performed at Invitrogen using P450 BACULOSOMES® reagents.

Table 3—$IC_{50}$ values (in µM) for CYP3A4.

| Compound | Testosterone 6β-OH assay | Vivid® BOMCC assay | Vivid® DBOMF assay |
|---|---|---|---|
| Ketoconazole | 0.04 | 0.1 | 0.02 |
| Mifepristone | 0.5 | 1.2 | 3 |
| Nifedipine | 3 | 0.7 | 0.3 |
| Omeprazole | 20 | 20 | 30 |
| Quinidine | 11 | 20 | 30 |
| Saquinavir | 2 | 2 | 3 |
| Sulfaphenazole | 500 | 200 | 400 |
| Testosterone | $K_m$ = 30 | activation | 45 |
| Verapamil | 1.0 | 3 | 9 |

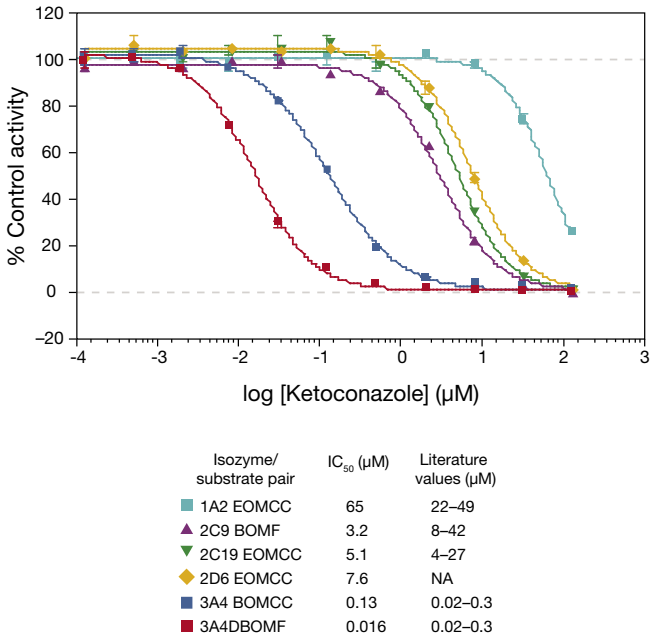



Figure 4—P450 screening results using the CYP3A4 selective inhibitor ketoconazole.

Take advantage of Invitrogen's efficient P450 Profiling Service. No subscription or enrollment is necessary. To get a project started:

1. Complete the P450 Profiling Submission Form (request by sending an email to P450profiling@invitrogen.com)
2. Receive confirmation from Invitrogen detailing project scope and pricing
3. Submit purchase order and prepared compounds to Invitrogen
4. Receive an electronic file of the results within 2 weeks

To learn more about P450 profiling services, visit www.invitrogen.com/P450profiling.

# Testing and profiling for nuclear receptor–mediated side effects

## PXR (SXR) and CAR—modulating the xenobiotic response

PXR (SXR) and CAR are known to mediate the expression of ADME-related proteins, notably certain members of the cytochrome P450 family and key drug transporters. Invitrogen has developed LanthaScreen™ time-resolved fluorescence resonance energy transfer (TR-FRET) assays to quickly assess test compounds for interactions with these important nuclear receptors.

Our LanthaScreen™ TR-FRET PXR (SXR) Competitive Binding Assay Kit* contains all the reagents needed to conduct compound affinity testing: a proprietary fluorescent Fluormone™ ligand, an optimized buffer system, a terbium-labeled anti-GST antibody, and purified PXR (SXR)-LBD protein. The PXR protein is indirectly labeled with terbium via the anti-GST antibody bound to the GST tag on the receptor. When this labeled protein binds to the Fluormone™ ligand, a high TR-FRET ratio will result. If a test compound that binds to the PXR protein is added to the reaction, the test compound will compete with the Fluormone™ ligand, resulting in a lower TR-FRET ratio. Since the change in TR-FRET ratio only occurs in the presence of a competitor, it can be used as a convenient and accurate indicator of the relative affinity of test compounds for PXR (Figure 5).

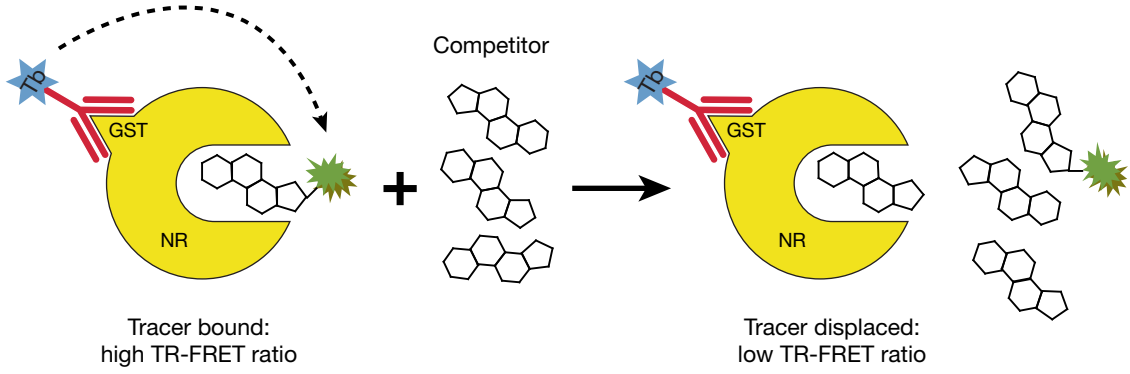



Figure 5—Schematic of LanthaScreen™ TR-FRET competitive binding assays.

* The PXR (SXR) protein is available from Invitrogen under an exclusive license from Puracyp, Inc.

Ligand binding to a nuclear receptor causes conformational changes in the receptor, resulting in a cascade of events including dissociation of repressor proteins, association of coactivator proteins, and assembly of Pol II and other transcriptional factors involved in the activation of target genes. A coactivator interaction assay has been developed for the study of CAR-LBD protein and the effects of test compound binding (Figure 6). The CAR assay relies on the conformational change in the receptor that takes place upon ligand binding, whereupon either the constitutive association of CAR with a fluorescein-labeled coactivator peptide is disrupted (by inverse agonists) or the interaction between the receptor and fluorescein-labeled coactivator peptide is enhanced (by agonists).

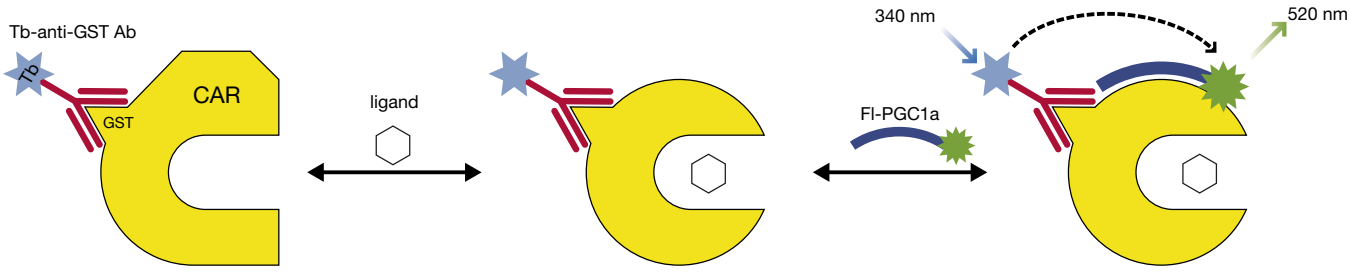



Figure 6—Principle of CAR agonist–dependent coactivator peptide recruitment.

Visit www.invitrogen.com/lanthascreen to learn more about LanthaScreen™ assay technology.

## A diverse panel of assays for your in-house profiling efforts

Invitrogen has developed a panel of human nuclear receptor proteins and assays designed to allow easy profiling of the following nuclear receptor targets (Table 4).

Table 4—Panel of nuclear receptor proteins and assays.

| Nuclear receptor | Isoforms | Protein | PolarScreen™ Competitive Binding Assay | LanthaScreen™ Competitive Binding Assay | LanthaScreen™ Coregulator Interaction Assay | GeneBLAzer® Live Cell Transcription Assay |
|---|---|---|---|---|---|---|
| AR | | ● | ● | | ● | ● |
| CAR | | ● | | | ● | |
| ER | alpha, beta | ● | ● | | ● | ● |
| ERR | alpha, beta, gamma | ● | | | ● | ● (no gamma) |
| FXR | | ● | | | ● | ● |
| GR | | ● | ● | | ● | ● |
| LXR | alpha, beta | ● | | | ● | ● |
| MR | | | | | | ● |
| PR | | ● | ● | | ● | ● |
| PPAR | alpha, beta, gamma | ● | ● (gamma only) | ● | ● | ● (no alpha) |
| RAR | alpha, beta, gamma | ● | | | ● | ● |
| RXR | alpha, beta | ● | | | ● | ● |
| PXR (SXR) | | ● | | ● | | |
| TR | alpha, beta | ● | | | ● | ● |
| VDR | | ● | ● | | ● | ● |

For a complete overview of these assays and techniques for nuclear receptor research, please visit www.invitrogen.com/nuclearreceptor.

## SelectScreen™ Cell-Based Nuclear Receptor Profiling Services—a flexible approach to screening

The SelectScreen™ Cell-Based Nuclear Receptor Profiling Service utilizes Invitrogen's rapidly growing library of GeneBLAzer® target-specific nuclear receptor cell lines and our robust GeneBLAzer® beta-lactamase reporter technology (Figure 7). This service offers a flexible approach to screening, enabling rapid profiling of your compounds against a panel of nuclear receptor cell lines by $EC_{50}$/$IC_{50}$ determinations in both agonist (% activation) and antagonist (% inhibition) modes using 10-point dose response curves.

This service can provide you with a wide range of combinations for screening—from a small subset of compounds against multiple cell lines, to many compounds against one cell line, such as a library screen.

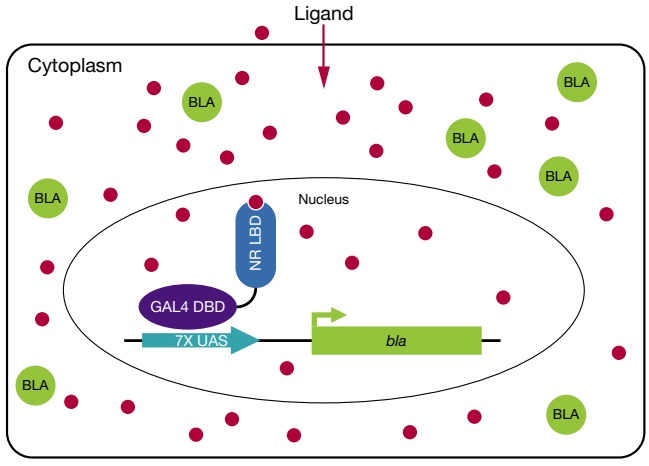



**Figure 7—Analysis of nuclear receptor activity using a GeneBLAzer® NR assay.** The UAS is activated by the GAL4 transcription factor DNA-binding domain (GAL4 DBD), which is expressed as a fusion protein with the target receptor ligand-binding domain (NR LBD). Upon ligand binding, the GAL4 DBD-NR LBD fusion protein translocates to the nucleus where it binds to the UAS, which controls transcription of beta-lactamase.

Visit us at www.invitrogen.com/NRprofiling to learn more.

# hERG cells by the plate for peak results

Regardless of their effectiveness at blocking target ion channels, all drugs must avoid blocking one channel: a cardiac potassium channel termed hERG (human ether-a-go-go related gene). Invitrogen now provides hERG-expressing cell lines for high-throughput patch clamping (HTPC) or traditional electrophysiological methods.

The hERG potassium channel is expressed in the mammalian heart and is crucial for repolarization and relaxation of cardiac muscle during every heartbeat. Potassium efflux occurs when the channel is open, and the cardiac myocyte membrane potential is positive with respect to the equilibrium potential for potassium (roughly –90 mV).

Mutations in this gene increase susceptibility to QT-interval prolongation, as shown by electrocardiogram readouts. This prolonged interval can lead to lethal ventricular arrhythmias. Carriers may be asymptomatic until a sudden startle stimulus (e.g., an alarm or an unexpected telephone call) causes fainting (if awake) or sudden onset of ventricular arrhythmia. Mutations typically decrease the amount of protein expressed on the cell surface: i.e., they encode trafficking-deficient proteins. A diversity of drugs from widely differing chemical scaffolds block this channel. Patients admitted for QT-interval prolongation or ventricular arrhythmia are typically screened for medications; therapeutically relevant levels of prescription drugs have been shown in patch-clamp assays to block hERG channels expressed in recombinant cell lines. Such findings have led to the withdrawal of 10 to 20 marketed drugs, and a recommendation from the ICH that all Investigational New Drugs be tested in such patch-clamp assays to assess hERG block liability before they are administered to humans.

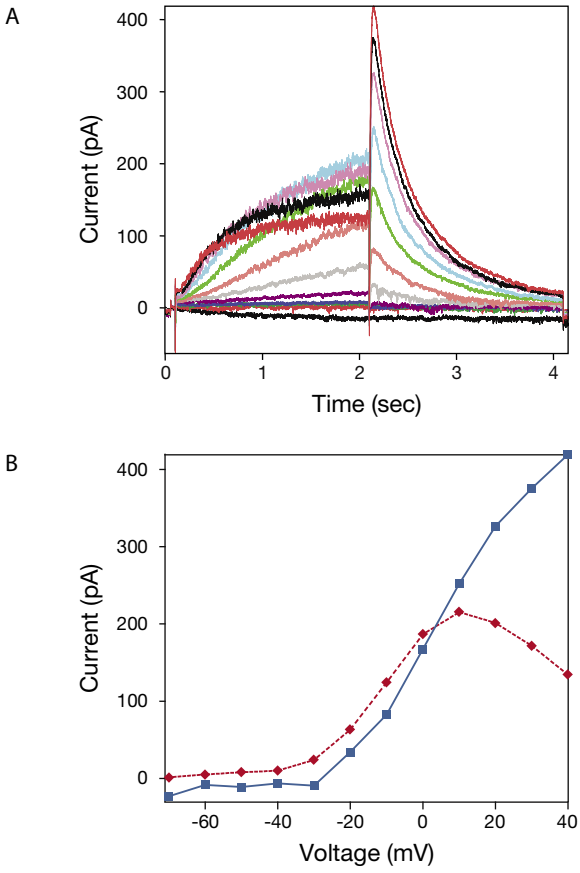



Figure 8—Currents recorded from hERG T-REx™ CHO cells in a patch-clamp assay. **A.** Cells were held at –90 mV and stepped in 10 mV increments from –70 to +40 mV, then back to –70 mV to elicit tail currents. **B.** The peak current for each step up (red diamonds) and the tail current for each step back to –70 mV (blue squares) are plotted.

We offer two cell lines utilizing Invitrogen's proprietary T-REx™ inducible expression technology, where there is little or no expression of hERG current until doxycycline is added. Twenty-four to forty-eight hours after addition of doxycycline, large currents are obtained from a majority of the cells examined in traditional patch-clamp assays (Figure 8). The two host cell lines, CHO and HEK293, offer the researcher flexibility of host choice, and the inducible expression technology offers the ability to tune the level of expression to configure optimal assays. For more information on hERG cell lines, visit www.invitrogen.com/herg.

The HEK293 cell line is also available as Division Arrested (DA) cells for easy plate and assay experiments. Invitrogen's Division Arrest technology delivers high-performance frozen hERG cells in discrete quantities for convenient and cost-effective cardiac safety screening.

With hERG T-REx™ DA cells, you can:

- → Achieve higher performance with greater peak currents than cells in continuous culture; ensure fewer failed experiments due to below-threshold performance (Figure 9)
- → Decouple the labor- and time-intensive live-cell production to improve your efficiency, consistency, and throughput
- → Obtain division arrested frozen cells in discrete quantities for convenient, cost-effective cardiac safety screening

DA cells are derived from a single clone of dividing cells as a result of a low-dose treatment with mitomycin C. This causes no apparent toxicity or change in cellular signal transduction. These cells have also been pretreated with doxycycline prior to division arrest, ensuring that hERG expression levels are optimal and that the cells are ready for immediate use.

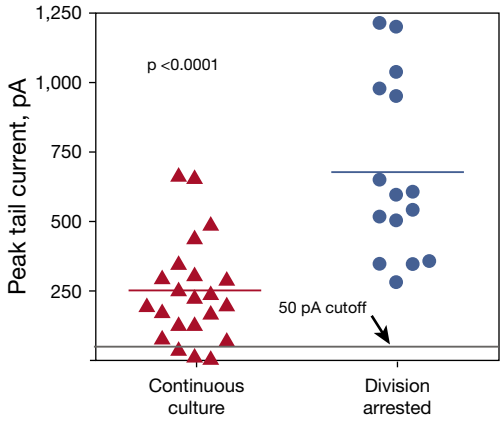



Figure 9—Currents recorded from hERG T-REx™ DA (Division Arrested) 293 and continuous culture hERG T-REx™ 293 cells using a patch-clamp readout.

Visit www.invitrogen.com/hergDA to learn more about hERG Division Arrested (DA) cells.

# Avoid off-target effects with our GPCR toxicity panel

With today's large compound library sets, primary screens can yield thousands of active compounds, so target selectivity and compound liability profiles are important in decisions to advance lead compounds. Targeting the correct receptor and avoiding off-target effects is essential. In addition to profiling within receptor families, a broader profile against receptors that are current pharmaceutical targets or known targets of drugs of abuse may be desired in order to identify additional potential addiction or side-effect liabilities.

Invitrogen offers a broad panel of well-validated cellular assays for GPCR selectivity screening (Table 5) in addition to assays against established drug target receptors. These cellular assays have been built using our GeneBLAzer® beta-lactamase technology, and are available either with dividing cells or in our new Division Arrested (DA) format.

Invitrogen's Division Arrest technology for GPCRs provides frozen cells by the plate for convenient, cost-effective screening. GeneBLAzer® GPCR Division Arrested (DA) cells can be assayed within 24 hours of thawing, and assay consistency can be

Table 5—GPCR liability panel.

| | | |
|---|---|---|
| 5HT1a | CRHR2 | EDNRB |
| ADORA2A | D1 | H1 |
| ADRA2A | D2 | H2 |
| AVPR1A | EDG3 | HTR7 |
| CCKBR | EDG7 | M1 |
| CNR1 | EDNRA | M4 |
| CRHR1 | | VPAC1 |

maintained over periods of up to five days. Because GeneBLAzer® DA cells exhibit response profiles similar to those obtained with our GeneBLAzer® dividing cells (Figure 10), you can be confident in the pharmacological data. In addition, cell numbers for division arrested cells increase only marginally after plating, thereby removing the variability caused by cell division during the course of an assay and providing more consistent results for day-long screening (Figure 11). GeneBLAzer® GPCR DA cells are stable cell lines derived from our fully validated GeneBLAzer® dividing cells that have been treated with a low dose of mitomycin C. This proprietary treatment causes no apparent toxicity or change in cellular signal transduction. GPCR DA cells are functionally validated using the GeneBLAzer® beta-lactamase readout to determine Z´-factor and $EC_{50}$ concentrations using a primary agonist (data available upon request).

Visit www.invitrogen.com/GPCRassays for a complete list of GeneBLAzer® GPCR cell lines.

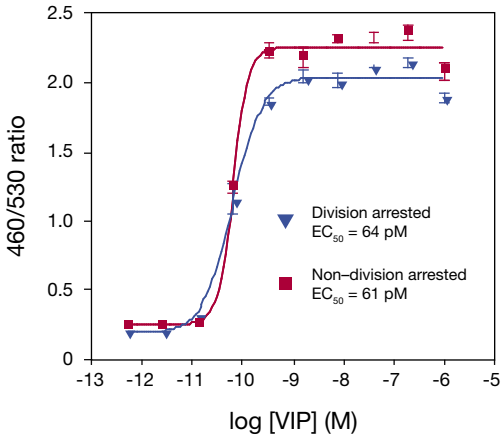



Figure 10—Dose responses to VIP of VPAC1 CHO-K1 non–division arrested and division arrested cells show similar $EC_{50}$ values.

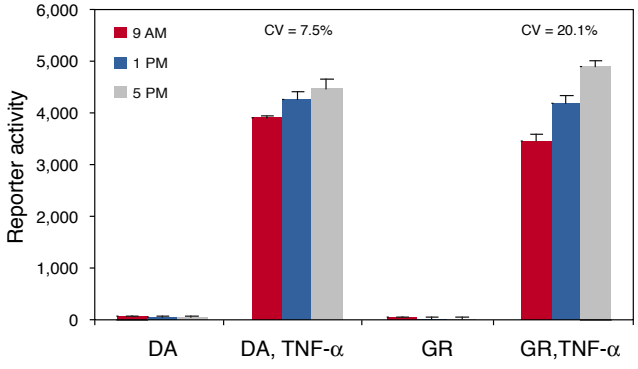



Figure 11—Improve assay consistency with division arrested cells. Three sets of division arrested (DA) and growing (GR) cells were plated at the same time but entered the assay at three different time points. Better %CV and Z´-factors were observed with division arrested cells (CV 7.5%, Z´-factor 0.76) than with growing cells (CV 20.1%, Z´-factor 0.38).

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The HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kits deliver readouts on two facets of cytotoxicity: the intracellular accumulation of phospholipids and of neutral lipids. Phospholipidosis detection is achieved using a green or red fluorescently labeled phospholipid; steatosis detection is achieved with a proprietary neutral-lipid stain. These kits are designed for fixed-endpoint workflows in which formaldehyde-fixed cells in microplates are processed, imaged, and analyzed.

Visit www.invitrogen.com/hcs for more information on these products.

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The LIVE/DEAD® Viability/Cytotoxicity Kit for animal cells provides a simple two-color fluorescence-based method for determining viability or assaying cytotoxicity of cultured cells, including adherent cells and certain tissues. The kit contains two probes, calcein AM and ethidium homodimer-1, which measure two recognized parameters of cell viability: intracellular esterase activity and plasma membrane integrity. Calcein AM is a fluorogenic esterase substrate that is hydrolyzed intracellularly to a green-fluorescent product (calcein); thus, green fluorescence is an indicator of live cells. Ethidium homodimer-1 is a high-affinity, red-fluorescent nucleic acid stain that is only able to pass through the compromised membranes of dead cells.

Calcein and ethidium homodimer-1 can be detected using standard fluorescein and propidium iodide filter sets, respectively. The LIVE/DEAD® Kit is designed for use with flow cytometers, fluorescence microscopes, and fluorescence microplate readers, and can be adapted for use with fluorometers.

To learn more visit www.invitrogen.com/hcs.



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