eurofins | DISCOVERY

EFFICIENT HIT-TO-LEAD FINDING CAPABILITIES FOR POTENT AND SELECTIVE BIOLOGICAL OUTCOMES

Reliable Chemistry, Target-based and Cellular Functional Services, and Products to Help You Design Successful and Fully Tailored Screening Cascades

Eurofins Discovery.
Deep resources for success.

Overview: Key Tools for Designing Successful Fully Tailored Screening

Identifying biologically and pharmacologically active compound series with desired potency, selectivity, and ADME-toxicology properties in early phases of drug discovery requires team expertise across multiple disciplines, including chemistry, high-throughput screening (HTS), ADME, safety pharmacology, toxicology, bioinformatics, automation and translational biology.

Eurofins Discovery delivers experts in those key scientific disciplines, with more than 40 years of experience in drug discovery, and more than 600 scientists fully skilled in testing, assay development, and project management. Our scientists complement and augment your team with a comprehensive range of fully validated best-in-class Discovery Services and DiscoverX® products. Through access to our innovative and breakthrough technologies, as well as hit generation and chemical lead identification strategies, you can deliver life-changing therapies.

Our operational, technical, and project management excellence provides you flexibility to select the most appropriate approach for your needs, from stand-alone assays to tiered HTS cascades and fully integrated discovery programs.

Our contract research expertise and depth of knowledge in preclinical development mean clients trust us—with new chemical entity development, testing for biological function and safety, plus guidance on selecting effective and safe drug-like characteristics from the earliest stages of discovery.

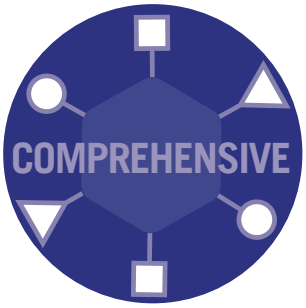
Step Inside to See How We Can Work Together

Our partnering process offers multiple advantages to help clients find their lead molecule faster

Established track record of high quality drug discovery services & products

Recognized as a trusted service provider >1,000 clients globally

Global team of scientific, operational, commercialization and client support professionals with diverse & specialized skills



One of the industry's largest and broadest drug discovery portfolios of services & products

Adaptable study designs for standard assays & customized assay development

High-quality reproducible data with appropriate controls in every client study

Learn more at eurofinsdiscoveryservices.com and discoverx.com

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HIT GENERATION CAPABILITIES AT A GLANCE

At Eurofins Discovery, we provide a comprehensive range of testing services and products for preclinical hit generation. From chemistry and biology to integrated drug discovery and project management, you will find our team to be the best match to propel your work forward.

40+ years of experience in contract research drug discovery and preclinical development

30+ billion reliable data points delivered to clients

600,000+ compounds available as a set of screening collections, for library enrichment

4,500+ off-the shelf assay services validated for *in vitro* pharmacology

3.0 billion compounds in 1 virtual screening library

50+ human primary-cell based models of tissue and disease biology

7,500+ ready-to-use assays, cell lines, and reagents

300+ genetically-characterized human cancer cell lines with 100 spheroid cell line options

Chemistry: Access synthetic and medicinal chemists; expertise in computational chemistry; flexible business models from full-time equivalent (FTE) based to FlexLab Custom Chemistry projects or insourcing of chemists;

Libraries, Assays & Products: Screen collections for library enrichment; virtual library screening options; HTS-compatible compound management; rapid Hit-to-Lead with the industry's widest selection of target-based and cellular functional ready-to-use tools;

DiscoveryOne™: Benefit from seamless project management, design, and execution capabilities; manage hit identification, triage, and validation programs under one roof.

Learn more at eurofinsdiscoveryservices.com and discoverx.com

CHEMISTRY

SYNTHETIC AND MEDICINAL CHEMISTRY SOLUTIONS

Our chemistry experts and modern best-in-class laboratories provide access to high-quality collaborative synthetic and medicinal chemistry capabilities.

We deliver customized and rapid solutions to suit your time demands, across major therapeutic areas, gene families, and target types, solutions that include:

- Rapid execution on milligram to kilogram scale with expertise in a diverse range of compound classes and specialized chemistry, including heterocyclic drug-like small molecules, linear and cyclized peptides, natural products, peptidomimetics, nucleotides and macrocycles
- Quality preparative chiral high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) purification with flow, photoredox catalysis, electro-chemical, and microwave chemistry capabilities
- Faster "Design-Make-Analyze-Test" iterations with co-located high-throughput experimentation (HTE) and ADME testing capabilities
- Agile and personalized project management focused on hypothesis-driven and multi-parameter optimization that, combined with locations across the globe for communication and business continuity, advance your project with maximum efficiency.

Eurofins Discovery provides deep expertise in chemistry, with our scientists having already delivered 20+ compounds into the clinic.

300+
Patents and Publications

40+
Development Candidates

CNS
9 Projects

1 compound in Phase I for chronic pain

1 candidate in preclinical development for Parkinson's disease

Autoimmune
30 Projects

1 compound in Phase II for diabetes

1 compound in Phase IIa for diabetes and obesity

1 compound in Phase I for solid organ transplantation

1 compound in Phase I for psoriasis

Cardiovascular and Metabolic
19 Projects

1 compound in Phase IIa for hypertension

1 compound in Phase IIb for obesity

1 marketed drug

Antibacterial
5 Projects

1 gut restricted clinical candidate for IBD

1 compound in Phase I

1 compound in Phase Ib

2 marketed drugs

Oncology
13 Projects

4 compounds in Phase I

1 compound in Phase II

Gastrointestinal
4 Projects

1 compound in Phase I

Viral Infection (PPI-Cyclophilin)
4 Projects

2 candidates for HCV infection

CHEMISTRY...continued

COMPLEMENTARY APPROACHES TO HIT IDENTIFICATION

Eurofins Discovery offers comprehensive and complementary methods to identify novel hit chemical matter, whether through a medicinal chemistry-driven literature-to-lead approach, computationally supported virtual screening or biology driven fragment, or high-throughput screening, to establish multiple tractable hit series. These options can be selected individually or in parallel; and when combined with our ability to deliver a robust screening cascade to assess hit quality and tractability, they generate an enviable starting point for a Hit-to-Lead program.

COMPUTATIONAL CHEMISTRY AND VIRTUAL APPROACHES

For those interested in a computational-based approach, we have in-house expertise in computational drug discovery and virtual screening across most target classes and especially for GPCRs.

Our ability to search for promising leads in collections of over 3 billion compounds is possible in two modes:

- Structure-based approaches (X-ray and homology models), which use known or homology structures to search for potential target binders
- Ligand-based approaches (3D shapes/pharmacophores), which use known compounds as starting points to identify novel compounds that display similar 3D shapes and pharmacophores.

Once the search is complete, a selection of these virtual hits is purchased or synthesized and supplied within 4-5 weeks for testing and structure-activity relationship (SAR) assessment.

View the Webinar: Hit Discovery for GPCRs: HTS or Virtual Screens?
Carleton Sage, PhD, Vice President of Computational Sciences at Eurofins Discovery, discusses diverse and complementary hit discovery approaches from projects executed by Eurofins Discovery teams: an HTS campaign for the orphan receptor GPR174, and a massive virtual screen that includes validation of docking to the serotonin 2B receptor. youtu.be/iFrhgnoze-0

LIBRARIES AVAILABLE AT EUROFINS DISCOVERY

A dynamic master library of 600k+ compounds employing a computationally driven approach Library Expansion/Enhancement is available for your programs. Current curated libraries include:

- 50k Small Diversity library
- 50k Small GPCR-focused library
- 240k Large Diversity library
- 250k Large GPCR-focused library
- 2k+ Fragment library consisting of a complimentary combination of Eurofins Discovery and Liverpool ChiroChem (LCC) fragment libraries.

FOCUS: LARGE DIVERSITY LIBRARY

Selected for drug-like properties:

- Low numbers of compounds with PSA, MW and Log P outside drug-like space
- Low percent of compounds with associated pan-assay interference compounds (PAINS) or Dundee Flag (6%)

Focus of this main screening library is on diversity of chemical space:

- No single large cluster at any given profile
- 7 clusters containing only one or two compounds
- 7 clusters with over 200 members
- Continual enhancement to broaden diversity of chemical space

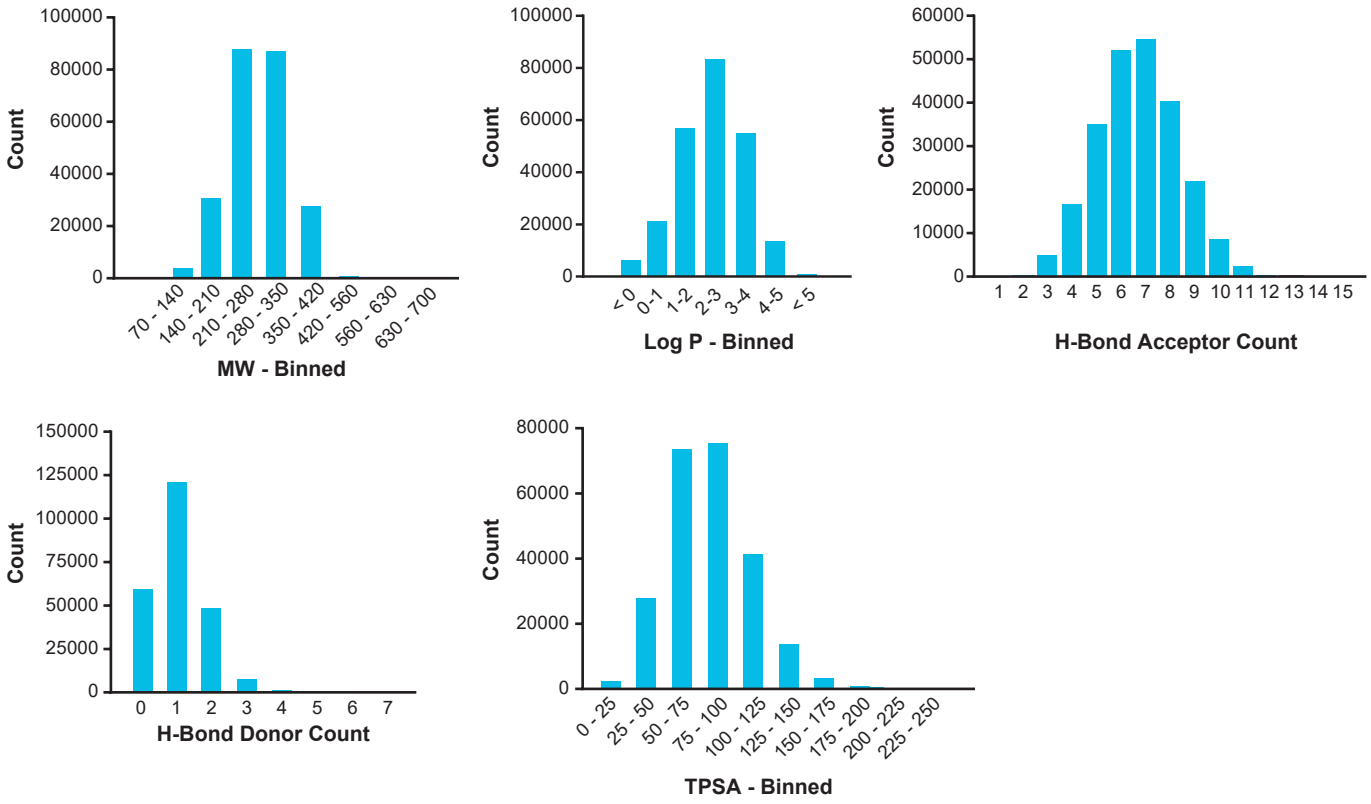


Figure 1. Key Physicochemical properties of the Large Diversity Library available from Eurofins Discovery.

Interested in having access to promising scaffolds? Learn more at eurofinsdiscoveryservices.com and Key Chemistry Capabilities

TARGETED BIOLOGY

OVERVIEW

With 4,500+ off-the-shelf, high-quality Discovery assays and 7,500+ ready-to-use DiscoverX® products, Eurofins Discovery covers the broadest range of targets and biology to match your hit identification and lead optimization needs, for both common and rare target classes and therapeutic areas.

Target class	Assay count	Assay type highlights	Technologies and expertise highlights
GPCR	2,750+	cAMP, Ca^{2+}, radiolabeled binding, β-arrestin, IP1, pERK, [^{35}S]-GTPγS, internalization, and PathHunter® Pharmacotrafficking cell-based assays	Radiometric, luminescence, fluorescence, mass spectrometry (MS), FLIPR® Tetra®, Enzyme Fragment Complementation (EFC), PathHunter, cAMP Hunter™ and TR-FRET assays
Kinases	1,000+	Functional, binding, activity, cell-based, and target engagement assays	qPCR, radiometric, luminescence, fluorescence, TR-FRET, InCELL Pulse™, InCELL Hunter™, NanoBRET™, MS, EFC and surface plasmon resonance (SPR) assays
Ion channels	400+	Binding, automated patch-clamp, and dye-based assays, microelectrode arrays (MEA)	Radiometric, IonFlux High Throughput (HT), QPatch, IonWorks Quattro systems, conventional manual patch clamp electrophysiology, HT, Qube 384 automated patch clamp (APC) and FLIPR Tetra assays
NHRs	150+	Protein-protein interaction, nuclear translocation, and cell-based assays	Luminescence, EFC, and TR-FRET assays
Interleukins / Checkpoints	50+	Signaling assays, dimerization	Luminescence and EFC assays
Epigenetics	160+	Binding, activity- and cell-based assays	qPCR, radiometric, EFC, InCELL Hunter, fluorescence, TR-FRET and MS assays
Toll-Like Receptors (TLRs)	20+	Cell-based inhibition and activation assays	Luminescence assays
Other enzymes and targets	100s+ combined with assay development	Custom assay development capacities to above-described plus multiple new innovative technologies	Cell line establishment combined with radiometric, luminescence, fluorescence (TR-FRET, Fluorescence Polarization (FP)...), EFC, FLIPR, Qube 384 APC, RapidFire® HT MS (RF-MS) and SPR assays

Table 1. Overview of the breadth and depth of target-specific testing services available from Eurofins Discovery and off-the-shelf assay products from Eurofins DiscoverX. Additionally available, DiscoverX custom assay development and Discovery test development capabilities are also available to better meet needs of small molecule discovery teams.

Through investments in state-of-the-art platforms and technologies, our R&D team develops products (e.g., modified cell lines, recombinant active proteins, cell-based functional assays), as well as binding and translational assays suitable for efficient high-throughput hit finding approaches combined with user-friendly reports and dedicated project managers' insights for actionable data.

Assay type	# Plates / day	Approx. # data points / day
Cellular functional assays	50-200	65,000
Ion channel Qube assays	10-40	4,000-16,000
Enzymatic activity assays	280	90,000
Enzymatic binding assays	150	55,000

Table 2. Examples of high-throughput testing capabilities available at Eurofins Discovery sites.

OUR COMMITMENTS TO OUR CLIENTS

Hit Identification

- Procurement and production of reagents, recombinant proteins, cell lines and assay kits for screening cascades
- Assay development and validation, including automation and miniaturization, for primary, secondary, and orthogonal assays
- Access to Eurofins Discovery's updated compound libraries as well as third party libraries
- Pilot studies for select on-target compounds

Hit Confirmation & Triage

- Confirm selectivity and mechanism-of-action (MOA) with secondary and orthogonal assays
- Determine chemical class druggability (reactivity, stability, solubility, synthetic feasibility)
- Triage early with cellular functional assays for toxicity and safety

Lead Validation & Optimization

- Determination of *in vitro* ADME properties (solubility, permeability, protein binding, clearance and lipophilicity)
- MOA insights with translational phenotypic assays and services
- Prediction and risk mitigation of potential off-target adverse effects and liabilities with safety panels
- Product kits similar to our lab-performed tests for you to run in your own lab facilities to complement your studies.

FOCUS: G PROTEIN-COUPLED RECEPTORS (GPCRS)

GPCRs play an important role in disease pathogenesis and constitute the largest family of transmembrane proteins in the human genome, making them one of the most attractive therapeutic target classes. In addition, 100+ GPCRs in the human genome are classified as orphan receptors, with endogenous ligands unknown, making GPCRs even more promising targets for drug discovery.

Support from Concept to Clinic

Eurofins Discovery is a dynamic leader in GPCR-based drug discovery, with a comprehensive range of solutions from stand-alone GPCR-specific DiscoverX® products and testing Discovery services to a fully integrated, comprehensive approach for the most challenging GPCR targets. With new GPCR-targeted candidates showing increased diversity (including biologics, allosteric modulators, and agonists for various disease indications), our more than 20 years of work in this critical target class brings key advantages.

Our strong reputation for GPCR drug discovery solutions stems from successful collaborations with biopharma companies around the world. To date, Eurofins GPCR experts have identified more than 30 preclinical candidates, and we have more than 20 programs progressing to clinical development.

For each project, we ensure:

- 2,700+ fully validated GPCR-related products and testing services
- Advanced discovery platforms and technologies
- In-depth evaluation of novel and orphan GPCR targets
- Design and deployment of the most effective screening strategies for your unique and evolving program needs
- Project management that delivers according to expectations and pace.

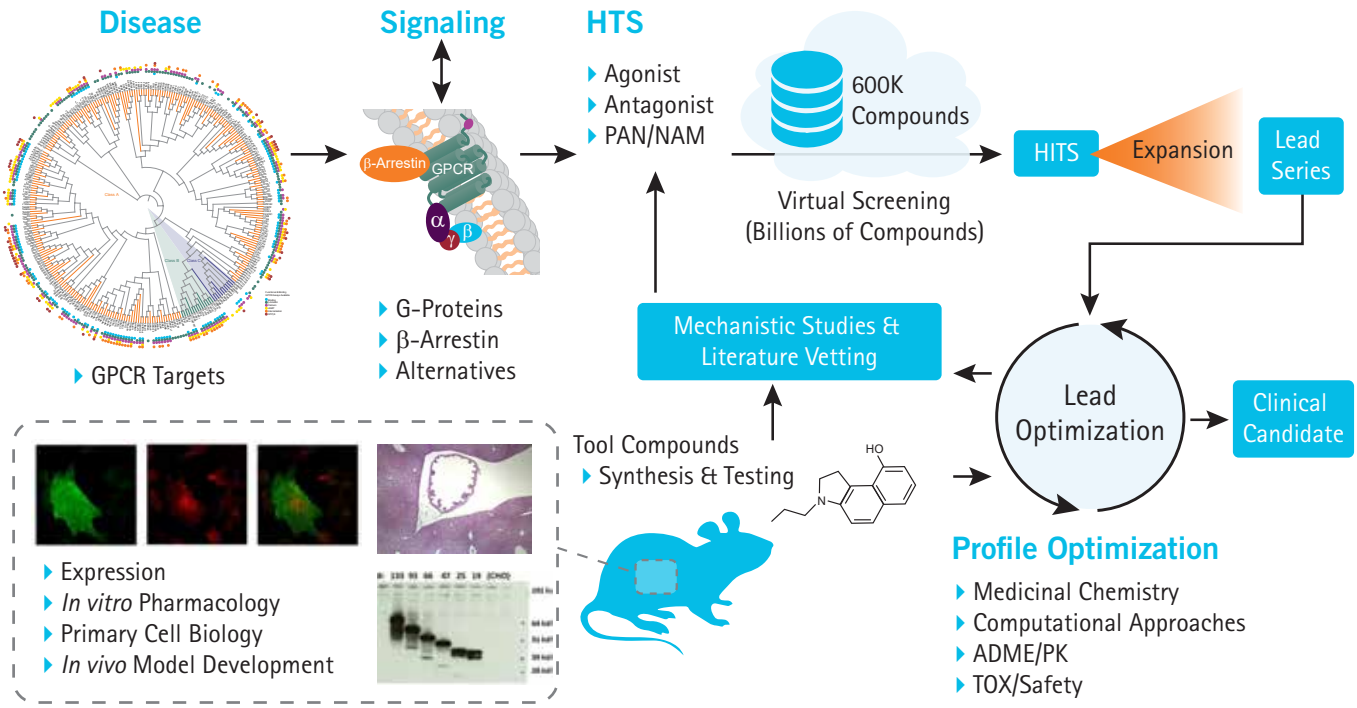


Figure 2. Schematic representation of the integrated workflow proposed by the Eurofins Discovery team to accelerate our partners' GPCR drug discovery programs.

Most Comprehensive GPCR Assay Collection in the Industry

Develop the optimal screening cascade with our unique collection of assays that saves months of assay development and testing time, maximizing your valuable resources. We support custom cell line and assay development, and deliver rapid turnaround times for efficacy, potency determinations, hit identification, selectivity profiling, and lead optimization.

- Choose from a comprehensive portfolio of off-the-shelf and fully validated GPCR-specific biochemical and cell-based assays
- Use a breadth of approaches to identify active compounds and allosteric modulators, including radio-ligand binding, β-arrestin recruitment, cAMP, IP-1, or calcium second messenger assays, internalization / trafficking assays and biophysical approaches
- Select the optimal technology for diverse readout needs, such as qPCR, luminescence, TR-FRET, EFC and FP assays, and for label-free biophysical readouts such as MS, SPR, and microscale thermophoresis (MST)
- Gain access to compound screening collections with Diversity or GPCR-focused libraries for immediate incorporation into an HTS campaign; as an option include your client-supplied library
- Screen up to 50k – 100k+ compounds per week with acoustics platforms for compound dispensing and reformatting, and automated robotic systems for efficient compound screening
- Reduce downstream attrition rates with proprietary platforms such as BioMAP® Diversity Early Screening for orthogonal assessments as well as selectivity, ADME, and safety pharmacology testing.

FOCUS: KINASES

With 80+ small-molecule kinase inhibitors currently approved by worldwide regulatory agencies for indications primarily in oncology and inflammation, and only 30% of the human kinome explored, numerous opportunities remain for this target class. Challenges remain as well, including: finding new therapeutically promising kinases; preventing acquired cancer resistance caused by mutations; accelerating first-in-class and CNS-penetrant next-generation kinase inhibitors; developing combination therapies; and exploiting polypharmacology versus optimizing selectivity. To meet today's kinase inhibitor development challenges, it is crucial to have access to a wide range of accurate, robust, and scalable *in vitro* kinase-based screening and profiling assays.

Over the last decade, Eurofins Discovery has developed a unique portfolio of kinase-based binding and functional assays with multiple readouts and technologies to support our research clients in the identification of small molecule leads. Our kinase experts can assist you in designing and executing smart kinase screening cascades that, combined with OncoPanel™ and BioMAP® Human Phenotypic Platforms for genomic and mechanistic studies, rapidly advance effective preclinical candidate development.

Eurofins Discovery kinase assay services and Eurofins DiscoverX® products compatible with Hit-to-Lead and Lead Optimization programs, including:

- 420+ human wild-type, active, inactive, and unactivated kinases (incl. 130+ tyrosine kinase assays & 50+ clinically relevant mutants)
- 2 pillars for Hit Finding to Lead Optimization
  - KINOME*scan*® for compound binding affinity testing and Kd measurement down to pM range with no ATP required
  - KinaseProfiler™, the original gold-standard radiometric platform complete with 21 lipid kinases and PIKK, ATM, ATR and DNA-PK (TR-FRET format) for direct measurement of substrate phosphorylation (IC_{50})
- 8+ possible assay formats for efficient cascade design (including PCR, radiometric, TR-FRET, DiscoverX ADP-Quest & Hunter Plus accumulation assays, ADP-Glo™, DiscoverX InCELL™ Target Engagement (TE) and NanoBRET™ TE and MS assays) for timely and data-driven decisions throughout kinase drug discovery and development
- Eurofins DiscoverX off-the-shelf recombinant kinases (also used in KinaseProfiler services) undergo strict quality control for the highest purity, specific activity, and lot-to-lot consistency.

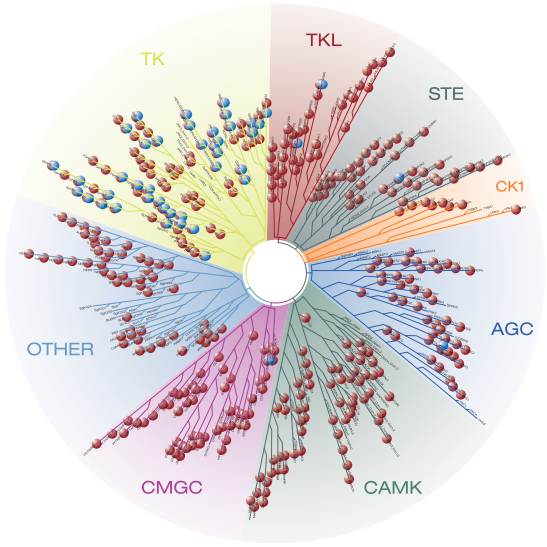


Figure 3. The Human Kinome.

CASE STUDY

HTS Cascade to Generate Highly Selective Hits for PIM3 Kinase

Context: The proviral insertion in Murine 3 (PIM3) proto-oncogene is a serine/threonine protein kinase playing critical roles in cancers and energy metabolism. Identification of new efficient and selective PIM3 kinase inhibitor scaffolds is therefore highly valuable for new therapeutic approaches in metabolic disorders and oncology including combinatorial therapies to prevent drug resistance.

Challenges: Kinase inhibitor discovery efforts are usually particularly challenging, because of conserved active sites and high structural homology between kinases leading to safety liabilities. Therefore, it is crucial to design hit-finding strategies based on high-quality enzymes and assay technologies to identify potent and selective lead compounds without compromising the development time of such programs.

Solutions: Eurofins Discovery HTS experts designed and executed a comprehensive and robust screening cascade to rapidly identify selective PIM3 inhibitors from Eurofins' proprietary libraries outlined by the screening cascade and the Gantt chart here (Figure 4.).

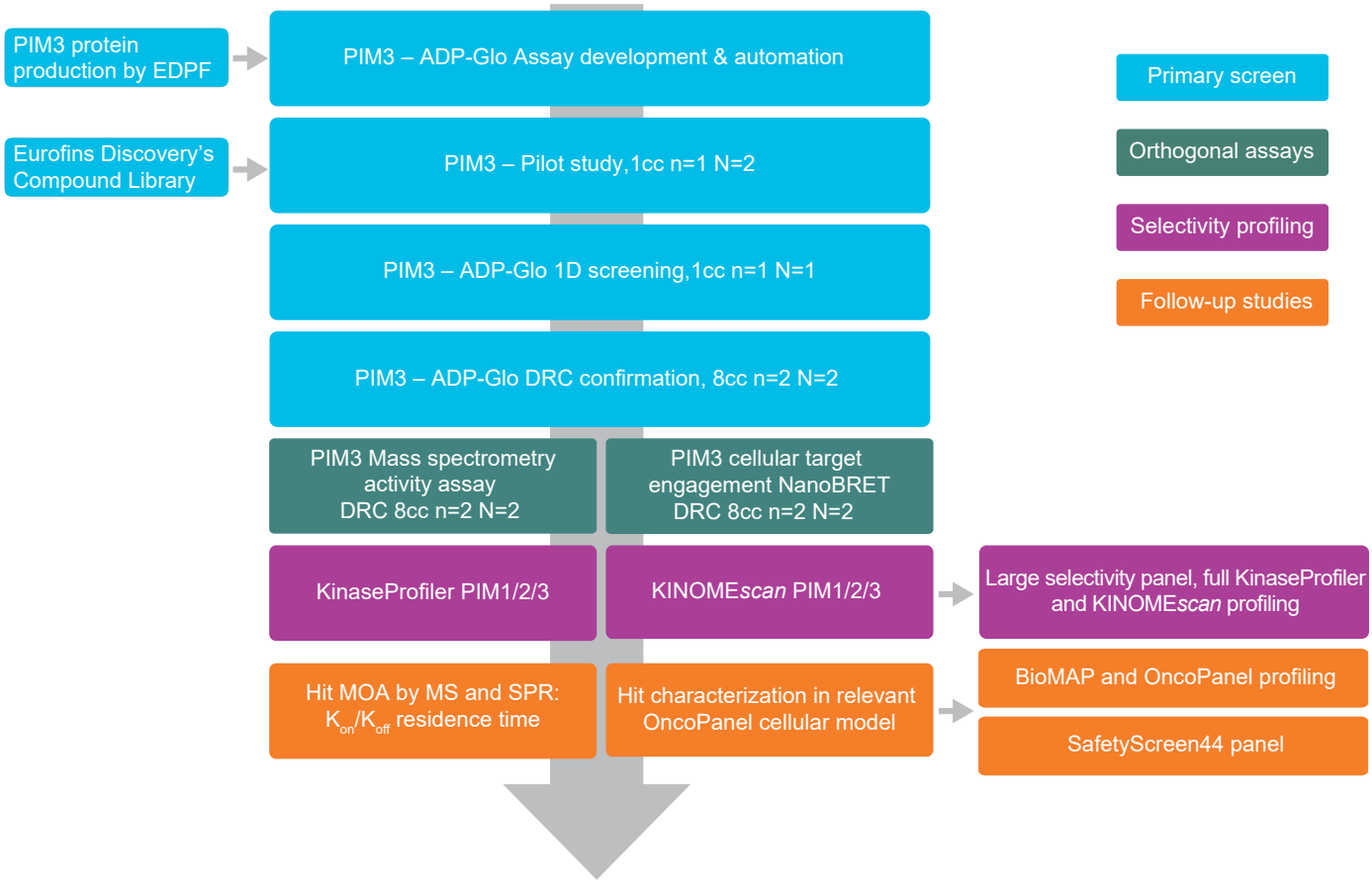


Figure 4. PIM3 screening cascade. A selection of Eurofins Discovery's Compound library (71k compounds in total) will be characterized using a PIM3-specific ADP-Glo™ assay during a pilot screen (one dose and dose-response curve (DRC)). Hits identified will be further characterized through activity MS-based assay and cellular NanoBRET™ TE orthogonal assays. Hit selectivity will be investigated against PIM1 & PIM2 kinases and the whole kinome using KINOME*scan*® and KinaseProfiler™. At a later stage, MOA of the hits will be investigated by MS and/or SPR assays, and their activity investigated in biologically relevant cellular models.

Standard Kinase HTS Gantt

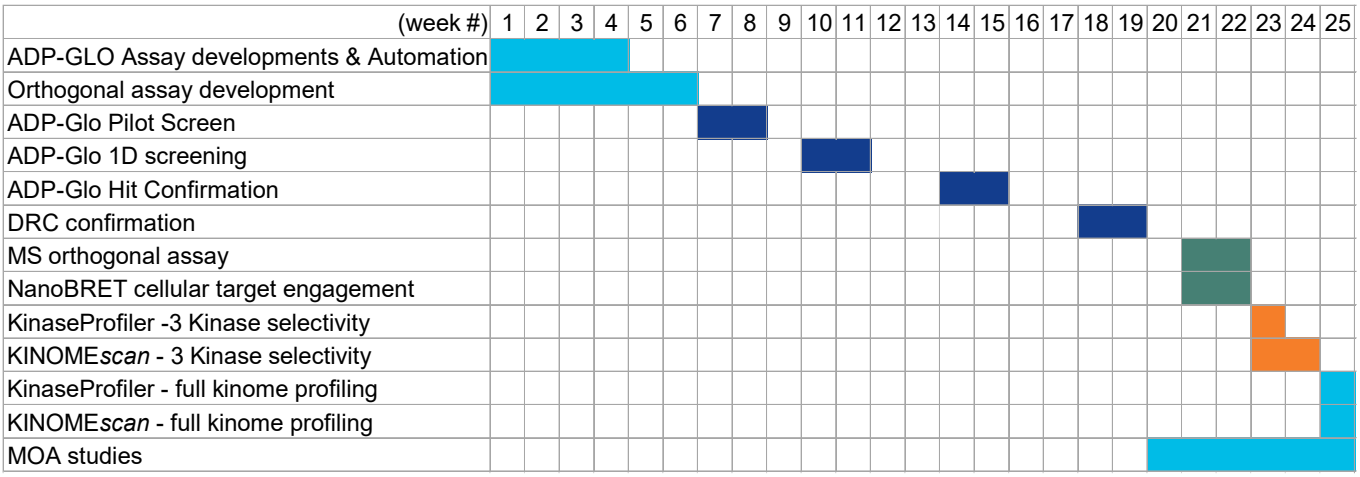


Figure 5. Kinase screening cascade Gantt chart. For a library up to 100k compounds, assay set-up can be performed in less than 4 weeks and hits identified and confirmed in less than 3 months, while fully characterized in orthogonal assays in less than 6 months from the initiation of the assay development (when compound resupply is required, time between phases could be expend to compound sourcing).

Results: High-quality recombinant active PIM3 kinase was produced in-house by our DiscoverX® experts to develop a robust high-throughput ADP-Glo™ primary assay used to screen the Diversity and Kinase Focus libraries (71k compounds). High-quality data sets (signal-to-noise ratio (SNR) & Z' monitoring, hit and confirmation rates) were obtained all along the cascade as depicted in Table 3.

HTS parameters	Pilot Screen	1Dose Screening	DRC
# Compounds	7,032	71,000	1,000
Testing conditions	1cc n=1 N=2	1cc n=1 N=1	8cc n=1 N=2
Average Z'	0.83 ± 0.06	0.86 ± 0.05	0.86 ± 0.03
Average SNR	39.6 ± 6.4	35.1 ± 2.7	27.4 ± 3.8
Selection Threshold (Mean + 3SD)	20%	31%	-
Hit Rate	1.32%	1.54%	-
Confirmation Rate	-	-	94.60%

Table 3. Monitoring of the quality indicators over the PIM3 screening cascade. Overview of the compounds tested and the conditions used in the different phases of the screening. The high quality of the data sets obtained are illustrated by the monitoring criteria (Z', Signal to Noise Ratio (SNR), hit & confirmation rates).

Demonstrated Capacities:

- Strong expertise of recombinant protein production and purification with DiscoverX, compatible with screening projects
- Scientific and project management know-how to establish and execute successful and robust screening cascade that combines multiple technologies and readouts
- Ability to deliver highly selective kinase inhibitors in just a few months, ensuring efficient Hit-to-Lead and lead optimization programs to produce valuable preclinical oncology candidates.

Learn more about this PIM3 Kinase hit-finding project at eurofinsdiscoveryservices.com and "HTS Generate High-Value Hits for PIM Kinase" Access the Application Note at eurofinsdiscoveryservices.com.

CASE STUDY

Characterization of Novel Inhibitors of Oncogenic SHP2 Variants Using Target Engagement Assays

Context and Challenges: A common challenge in drug discovery is the inability of many primary *in vitro* assays to properly discover and assess the cellular target engagement of candidate compounds in a way that corroborates with physiologically relevant models. The discovery and assessment of compound binding proves to be more challenging, particularly for targets such as SHP2 (Src-homology 2 domain-containing phosphatase 2) phosphatase, a key player in several signaling cascades such as the JAK/STAT, Ras-Raf-MAP kinase, and PIM3 kinase pathways.

Solutions: In a case study, Romero *et al.*[1] used InCELL Pulse™ cellular target engagement platform to not only screen but also characterize novel therapeutics targeting oncogenic forms of SHP2. The study focused on evaluating an array of allosteric SHP2 inhibitors against wild-type and an oncogenic variant (mutant E76K) using a 384-well miniaturized, high-throughput InCELL Pulse cellular thermal shift assay format.

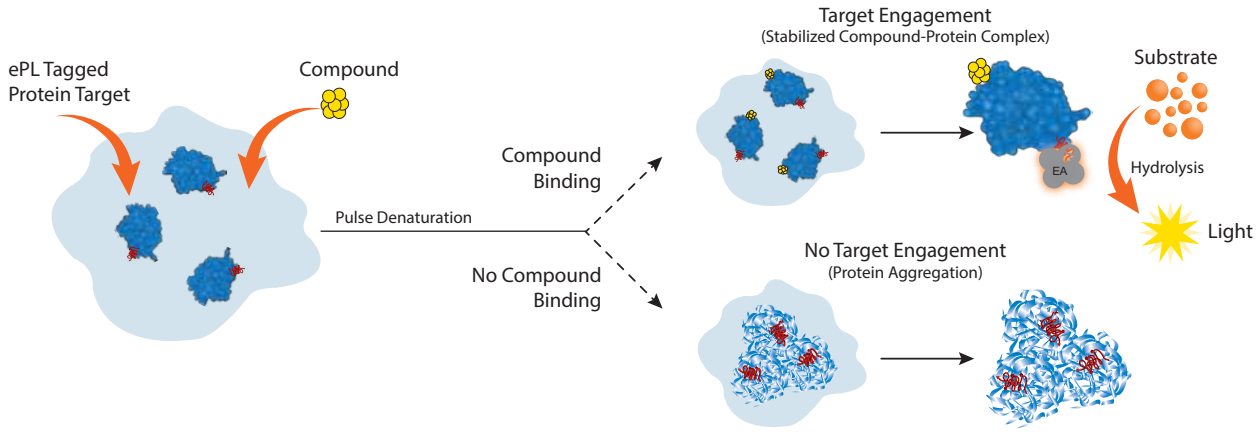


Figure 6. InCELL Pulse compound–target engagement assay principle. This cellular compound-binding assay is based on the novel Enzyme Fragment Complementation (EFC) technology incorporating the principle of protein thermal stability. Cells expressing a protein of interest fused to a small EFC donor enzyme fragment (called ePL) of a β-galactosidase enzyme forms the initial step. Cells are then treated with test compound and then subjected to elevated temperatures via a pulse denaturation step. Addition of a larger EFC acceptor enzyme (EA) fragment determines the fate of compound engagement, wherein binding of compound to target protein preserves the protein structure and enables complementation of EA with ePL, while non-binding of compound results in the denaturation of protein thus causing poor complementation of EA with ePL. Addition of a substrate allows for detection of strong or poor complementation between ePL and EA in the form of a corresponding chemiluminescent signal.

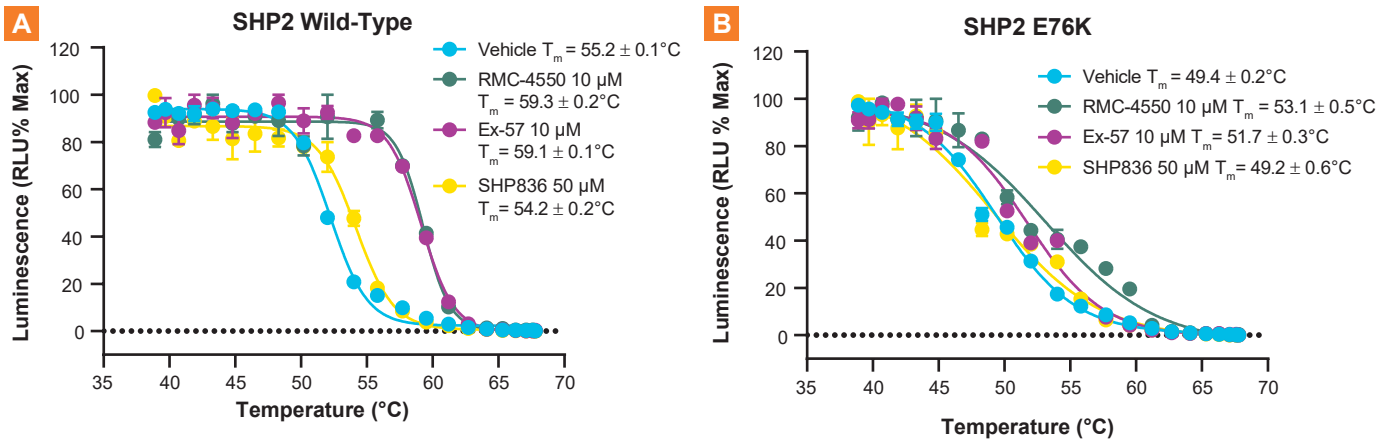


Figure 7. Thermal shifts of SHP2-wild type (WT) and an E76K mutant oncogenic variant from the InCELL Pulse cellular thermal shift assay. A. SHP2-WT displayed a superior thermal shift when treated with either Ex-57 or RMC-4550 inhibitors indicating successful compound-SHP2 binding. In contrast, SHP836 inhibitor had little detectable effect on SHP2-WT similar to the control (vehicle sample) B. All tested inhibitors caused dramatically smaller thermal shifts on the oncogenic E76K variant when compared to wild-type SHP2, indicating less compound-SHP2 binding possibly due to the mutation.

Results: This study focused on mutations that cause aberrant SHP2 activity, which is implicated in several cancers including leukemia and breast cancer. Abnormal SHP2 function has also been well-documented in 50% of Noonan Syndrome patients. Because of its role in multiple pathways and diseases, SHP2 continues to be a critical therapeutic target. Inhibiting SHP2 with orthosteric (or allosteric) small molecules has been challenging in the past and was analyzed in this case study using the InCELL Pulse™ thermal shift target engagement assay platform. Results revealed all tested inhibitors caused dramatically smaller thermal shifts on the oncogenic SHP2 E76K variant when compared to wild-type SHP2, indicating less compound-SHP2 binding possibly due to the mutation.

Additionally, thermal shift high-throughput screening was performed using 16 similar small molecule inhibitors to evaluate cellular compound binding to SHP2. Results (not shown here) indicated the assay is capable of high-throughput format, thus enabling easy lead ranking of many compounds quickly and efficiently in a cellular environment.

Such a finding has created a need for allosteric SHP2 inhibitors that selectively bind to disease relevant SHP2 variants. Since this discovery of allosteric SHP2 inhibitors, efforts to find similar therapeutics that successfully target SHP2 variants continues.

Demonstrated Capacities: Overall, the simultaneous evaluation of cellular permeability and compound binding is made possible with the InCELL Pulse assay platform. In early stages of drug discovery, not only is InCELL Pulse suitable for screening compounds, but it can also be used to analyze many targets whose function is not known. Thus, a broad range of targets (kinases, phosphatases, epigenetic proteins, proteases, PROTAC targets, ER and plasma membrane proteins, and more), full-length or otherwise, can be easily studied in a cellular context. With continuous efforts to help you accelerate your drug discovery programs, Eurofins DiscoverX® has developed different types of cell-based assays for your research needs.

[1]Romero, C., *et al.* (2020). *The Journal of Biological Chemistry*, 295(9), 2601–2613. doi.org/10.1074/jbc.RA119.010838

Access the application note at discoverx.com.

FOCUS: ION CHANNELS

At over 400 members representing about 1% of the human genome, ion channels are the third-largest class of therapeutically promising protein targets, after GPCRs and kinases. To date, more than 15% of all approved small molecule drugs are reported to modulate voltage-gated or ligand-gated ion channels in numerous applications (e.g., anti-arrhythmic and analgesic effects, anesthetics, treatments for epilepsy and seizures, as well as anxiety and insomnia).

With a fairly high amino acid sequence homology among the sub-members of the ion channel family, transport proteins are often considered challenging targets for drug discovery. Nevertheless, in recent years, ion channels including transport proteins have been the subject of many significant advances in the understanding of their structure, function and pharmacology. These advances have led to the emergence of new functional higher throughput assays and advanced technologies to implement more efficient pharmacological tools to evaluate channel modulators for hit identification (HTS), selectivity, profiling, and safety studies. These developments open new opportunities to discover and develop improved ion channel modulators that are more potent and selective for their primary target, with favorable pharmacokinetic profiles and fewer adverse effects.

Within this context, Eurofins Discovery provides an industry-leading portfolio of ion channel cell lines and technology platforms together with a dedicated team of experienced electrophysiologists to help you determine the right assay format (binding and/or functional) all the way through data interpretation.

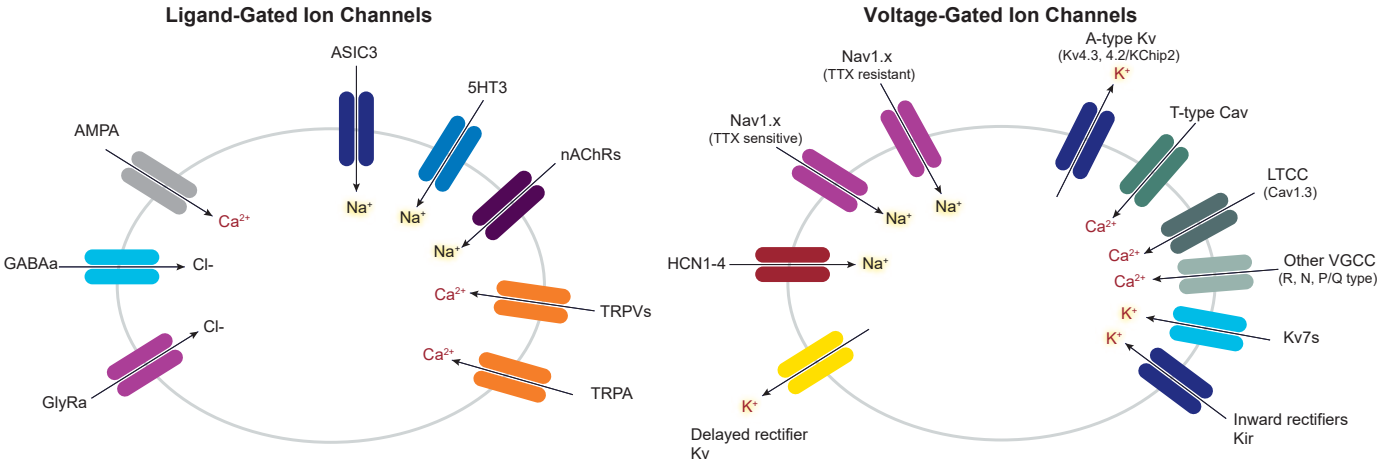


Figure 8. Eurofins Discovery HTS cell-based functional assays offer more than 60 ion channels to support drug discovery screening efforts.

From the gold standard single-cell manual patch clamp to high-throughput binding and flux assays, and recent 384-well automated patch clamp electrophysiology assays, Eurofins Discovery has the capabilities to use platforms and strategies to provide the throughput, results, and depth of analysis to meet your drug discovery requirements. A few examples:

- Functional Assays – Cell-based HTS functional assays for over 60 ion channels to support drug discovery screening efforts. Full project support, both scientific and technical, for ion channel drug discovery using HTS Qube384 automated electrophysiology platform technology as well as other supporting platforms
- Radio-ligand Binding Assays – For a number of ion channel families, including sodium, potassium, and calcium, Eurofins Discovery can perform radio-ligand binding assays using in-house produced membrane preparations to control the quality of these critical reagents and ensure reproducibility of data. A useful approach to monitor potential hERG liability in the earliest phase of drug discovery, where the primary objective is to raise signals that could trigger follow-up studies, such as electrophysiological screening
- Orthogonal and Follow-up Assays – Expertise in designing custom solutions from fluorescence and ion flow assays to ion channel cell line engineering and development
- Cardiac Ion Channel Safety Testing – HTS of hERG and other cardiac ion channel targets (Qube384 platform), as recommended by Comprehensive *in vitro* Proarrhythmia Assay (CiPA) collaborative network, for early cardiac risk assessment and mitigation
- PrecisION™ Ion Channel products – Fully-validated DiscoverX® cell lines and membrane preparation for use in cell-based functional assays, including manual and APC in fluorescent and luminescent platforms for ion channel modulators screening, safety pharmacology, hERG liability monitoring, and pharmacochaperone identification.

FOCUS: TARGETED PROTEIN DEGRADATION

Our protein degradation and proteolysis targeting chimera (PROTAC) discovery portfolio includes a comprehensive suite of target-based, cellular, and translational phenotypic approaches for a powerful drug discovery resource in this space.

Among these various approaches, E3scan Ligand Binding Assays accelerate screening and SAR analysis with rapid turnaround times for discovery library screens (20 business day TAT), weekly SAR studies (5 business day TAT), and the largest assay panel available on a single technology platform (e.g., VHL, MDM2, MDM4, CRBN, cIAP1 & 2 and XIAP).

The E3scan technology is particularly valuable to identify and characterize new, potent, and selective ligands that bind and reprogram E3 ligase substrate specificity. These assays enable sensitive and accurate ligand Kd assessments, down to low pM range, and are appropriate for quantitative competition binding assays.

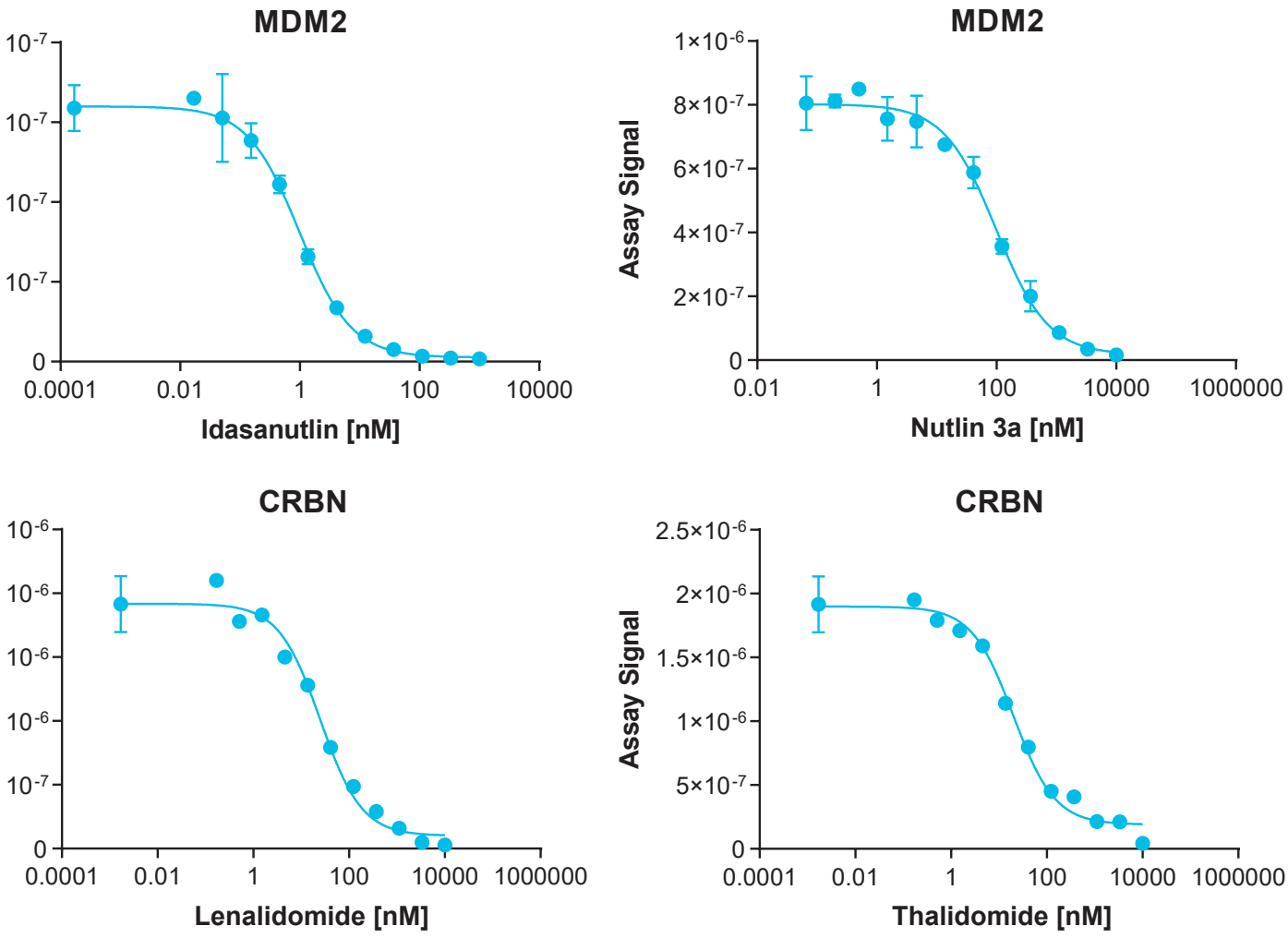


Figure 9. Representative data of MDM2 validation with two MDM2 Inhibitors (idasanutlin & nutlin 3a) and of CRBN with two CRBN inhibitors (thalidomide & lenalidomide).

TARGETED BIOLOGY...continued

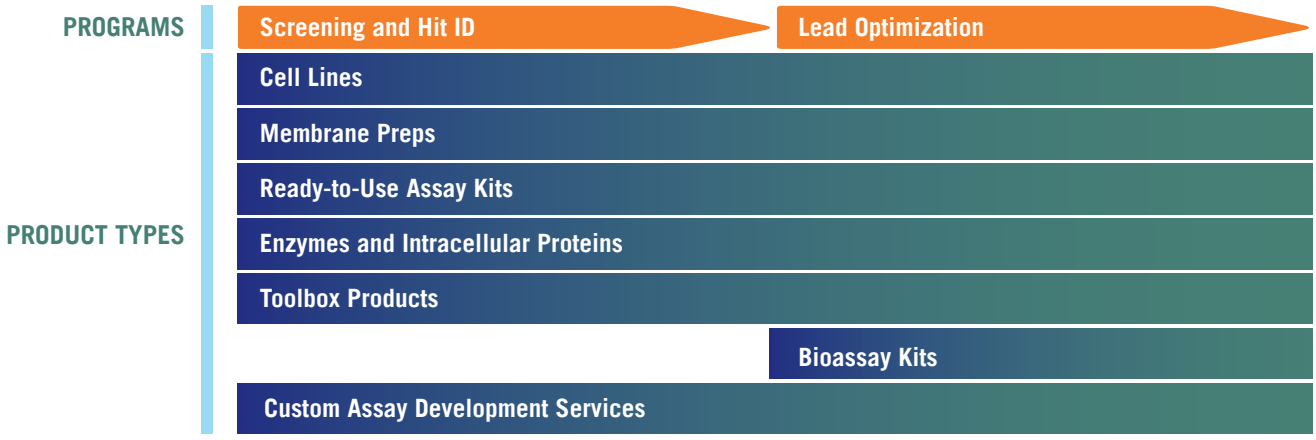
Additional Eurofins Discovery Services and DiscoverX® Products available for your protein degrader programs:

- Chemistry – Apply collective expertise in synthetic and medicinal chemistry to design oral drug-like properties into larger molecules in the 'beyond rule of 5' space
- KINOME*scan*®, BROMO*scan*, and BCL2scan – Develop, characterize, and validate the warhead end of novel PROTACs
- SPRINTer™ Protein Turnover Biosensor Assays – Enable sensitive quantitation of PROTAC-mediated degradation of targets with cellular biosensor cell lines
- BioMAP® Phenotypic Platform – Evaluate the impact of target degraders on efficacy and safety-related translational biomarkers in human cell-based disease models, or benefit from early SAR insights
- Safety Pharmacological Profiling – Fit-for-purpose panels for quick and accurate evaluation, prediction, and mitigation of potential risks at each stage of drug development in any targeted degradation program.

FOCUS: DiscoverX PRODUCTS FOR IN-HOUSE SCREENING

Whether you require outsourcing needs or perform research in-house, Eurofins DiscoverX® product solutions empower R&D through investigational new drug (IND) application. Easily and rapidly characterize targets, identify hits, optimize leads, perform compound-target engagement, and more:

- Robust cell-based assays support drug discovery and development programs in-house, from HTS through lead optimization and beyond
- Comprehensive product portfolio of cell-based functional and binding assay kits, cell lines, reagents, and recombinant purified proteins cover the top therapeutic target classes, including GPCRs, kinases, ion channels, checkpoint modulators, cytokine receptors, and nuclear hormone receptors.



Eurofins DiscoverX products include a broad portfolio of stable cell lines, validated functional and binding assays, complete ready-to-use kits, and optimized reagents developed and manufactured to support your drug discovery journey every step of the way.

Expedite your candidate through the drug discovery and development pipeline with Eurofins DiscoverX® assay products:

- Enabling Technologies – Improve productivity and effectiveness of your screening, lead optimization, and SAR campaigns
- Simple, Fast, and Scalable – Rapid, homogeneous, and simple mix-and-read assay format scalable from 96- to 3,456-well, and without washing, centrifugation, or filtration
- Robust Assays –MOA-reflective, cell-based assays with large signal-to-background ratio, high precision, and lot-to-lot reproducibility
- Qualified and Validated – Extensively optimized for hundreds of targets; used for screening billions of data points and cited in thousands of peer-reviewed publications

Comprehensive portfolio of products covering the top therapeutic target classes including GPCRs, kinases, cytokine receptors, checkpoint modulators, ion channels, and more.

Learn more at discoverx.com to start successfully screening today!

ADME, SAFETY, TRANSLATION

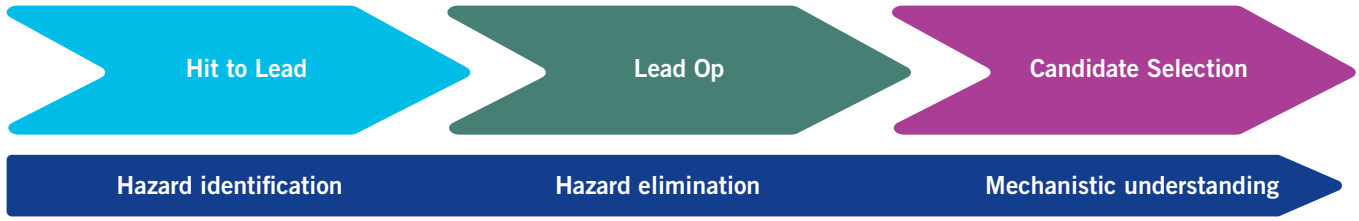
Early ADME and target safety assessment screenings are essential to prioritize compounds for development through long and costly clinical phases. Early identification of candidates with desirable metabolism and safety profiles inform SAR efforts and increase the likelihood of clinical success. Functional assays using translationally relevant biomarker readouts, including measures of cytotoxicity, provide a competitive advantage to effectively and efficiently select the best preclinical candidates.

PHYSICAL CHARACTERIZATION, SAFETY / TOXICITY ASSESSMENTS

Our portfolio includes cost-effective and comprehensive standard, or fully customized assay services for *in vitro* ADME, toxicology, secondary and safety pharmacology, as well as for *in vivo* models for non-GLP DMPK and safety.

This selection of essential assays and panels is organized through a tiered approach in the early phases of drug discovery to screen, identify, and select scaffolds with optimized and desirable pharmacokinetic properties. This accelerates candidate selection while anticipating or predicting safety liabilities.

HTS electrophysiology, human phenotypic profiling with the BioMAP® Platform, and *in vivo* safety models round out our safety offerings.



- Early hazard identification and off-target mitigation
- Design out potential liabilities and drive chemical design for SAR models
- Full mechanistic understanding of liabilities facilitates progress into first-in-human trials

Tier 1 ADME Panel	Tier 2 ADME Panel
A panel of 8 assays to determine compound solubility, permeability, protein binding intrinsic clearance and lipophilicity in 10 days	41 IND-enabling assays in one panel to provide comprehensive drug metabolism, transporter, and drug-drug interaction data necessary to progress lead compounds to preclinical development in 20 days
In Vitro Toxicology Panel	
24 toxicology assays to effectively de-risk drug candidates for a broad range of potential Adverse Effects (AEs) including hepatotoxicity, cardiac safety, genotoxicity, cytotoxicity, and renal toxicity in 15 days.	

Tier 1 Safety	Tier 2 Safety	Tier 3 Safety
Target-based Safety panels based on binding & functional assays		
SafetyScreen™ 44 Panel	SafetyScreen 87 functional Panel	SpectrumScreen® panel
SAFETY*scan*® 47 functional panel	Custom panels	BioPrint panel
Phenotypic & Comprehensive *in vitro* Proarrhythmia Assays (CiPA)		
CardiacProfiler CiPA core panel (hERG, hNav1.5 peak, and hCav1.2)	CardiacProfiler core Panel (hERG, hNav1.5 peak, hCav1.2, Kv4.3, KCNQ1/KmINK, Kir2.1)	
hiPS cell-derived cardiomyocytes FLIPR screen	hiPS Cell-derived cardiomyocytes FLIPR® DRCs	
	hiPS Cell-derived cardiomyocytes MEA screen	hiPS Cell-derived cardiomyocytes MEA DRCs

A versatile and evolving *in vitro* ADME and safety offer with a tiered approach in the early phases of drug discovery to screen, identify, and select scaffolds with optimized and desirable pharmacokinetic properties to accelerate candidate selection while predicting safety liabilities and mitigating risks.

PHENOTYPIC APPROACHES FOR A BIOLOGY-FIRST STRATEGY

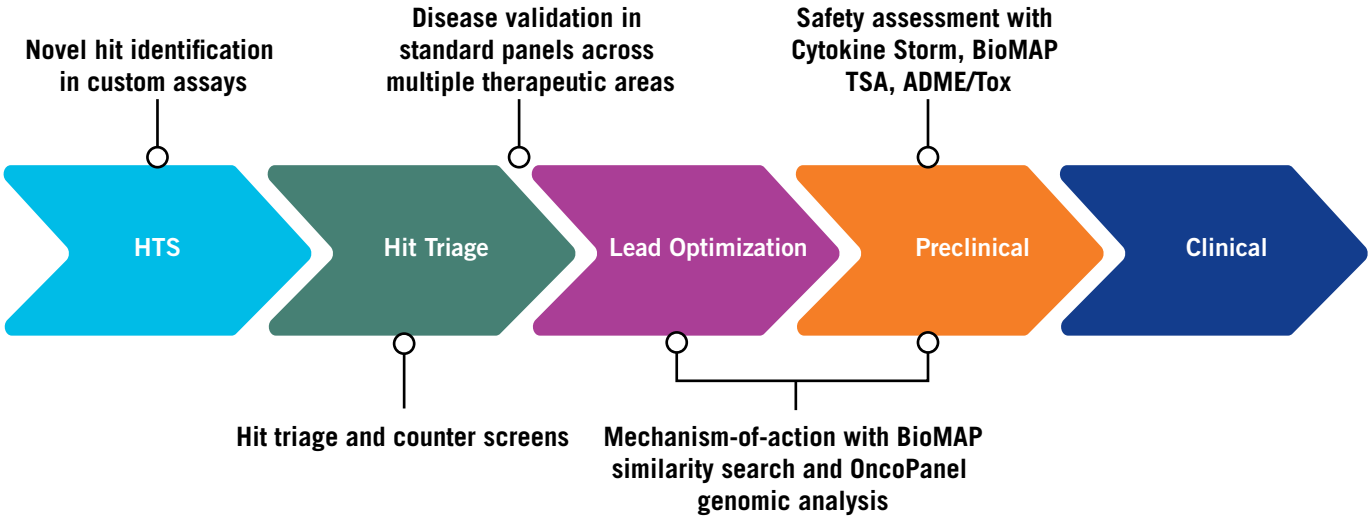
Translational phenotypic assays are a valuable tool for hit validation and qualification downstream of high-throughput primary assays and upstream of resource-intensive animal studies. Easily incorporated into a HTS drug development strategy, phenotypic screening and profiling positively guides medicinal chemistry efforts and provides an orthogonal approach to help evaluate series of related molecules in validated and translationally relevant assays.

Our Phenotypic Center of Excellence offers the robust OncoPanel™ and BioMAP® Platforms to ensure greater efficiencies in drug discovery from compound prioritization, indication selection, and mechanism-of-action (MOA) determination, to safety assessment and asset evaluation.

- OncoPanel provides 300+ immortalized human tumor cell lines, licensed from renowned repositories, for a breadth of *in vitro* models to use in cancer drug candidate screening
- BioMAP provides 50+ validated human cell-based disease models, curated into broad or therapeutic area disease panels, with translational readouts and predictive analytical tools to screen candidates for biological activities and toxicities.

Utilize these proprietary cellular functional approaches, validated with clinical drugs, to:

- Screen compound sets for rapid identification of hits from chemical series or target-specific collections
- Discriminate hits with novel biology
- Prioritize for efficacy-relevant biology, cellular activities, and therapeutic superiority
- Triage early for safety-related liabilities
- Identify safety concerns or potential adverse outcomes based on key biomarker activities in a BioMAP profile. Toxicity signatures detected include Acute Toxicity, Immunosuppression, Skin Irritation, Organ Toxicity, Skin Rash, Skin Sensitization, Thrombosis and Vascular Toxicity.



For preclinical programs, phenotypic assays provide benefits during HTS, compound triage, MOA, safety assessment, and hit prioritization. Shown are key phenotypic approaches offered by Eurofins Discovery, appropriate for different stages of the drug discovery pipeline.

Learn more at eurofinsdiscoveryservices.com and discoverx.com.

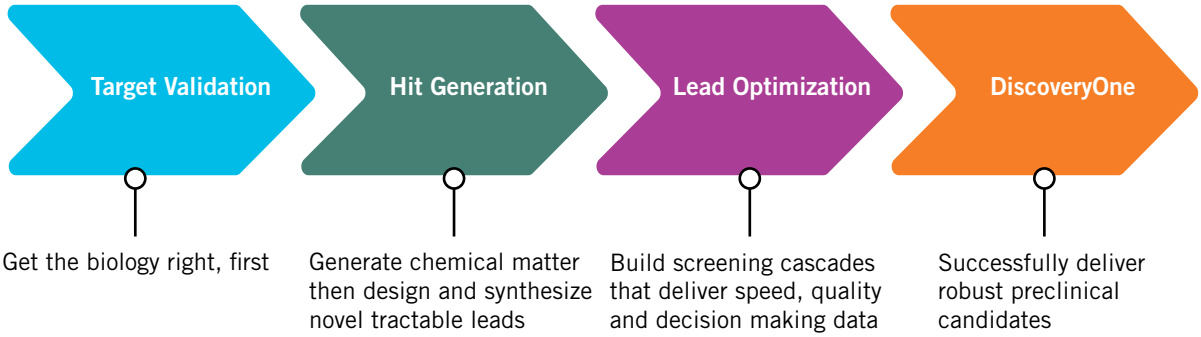
BENEFITS OF CHOOSING EUROFINS DISCOVERY FOR YOUR HIT-TO-LEAD DISCOVERY

Simply put, through decades of experience, quality, and flexible solutions, we accelerate drug discovery programs. If you have a promising molecule or target area of interest, but need to manage and coordinate multiple CROs in a complex process, a Eurofins Discovery collaboration can provide you the efficiency of one single point of contact. With our experienced team managing project complexities, your discovery is ensured to reach the finish line.

ACCESS TO INTEGRATED DRUG DISCOVERY SOLUTIONS

DiscoveryOne™ integrated drug discovery solutions combine our chemistry, biology, and technologies with our exceptional program management services.

Your personal DiscoveryOne project team will design and implement a comprehensive screening program to provide you with the data you need to advance the right therapeutic candidates. Working with DiscoveryOne gives you access to comprehensive services from assay-ready development to *in silico*, synthetic and medicinal chemistry, HTS to Lead Op and *in vitro* safety pharmacology, translational phenotypic assays, and ADME-tox. Whether you're looking for a one-off project or guidance through your entire drug discovery journey, Eurofins Discovery can be with you every step of the way. Our process:



- Start with validated HTS services chosen from our 600k+ proprietary compound collection, or leverage our computational chemistry capabilities to virtually screen our 3-billion-fragment library
- Follow up the study with any of our 7,500+ off-the-shelf assays for phenotypic screening, cellular or biochemical characterization
- Progress your program with confidence by directly working with our scientific experts who will let you know the minute something unexpected occurs and provide guidance to readjust your project onto the critical path.

Depth of Experience Combined with Breadth of Capabilities to Meet Your Requirements

- 600+ scientists at your side helping you design and execute the most appropriate screening cascades (including technicians, project managers, chemists, biologists, assay developers, engineers and roboticists)
- 9 Centers of Excellence across the globe with proven experience in assay testing and production of reliable products for HTS
- 7 main therapeutic areas (expertise in oncology, immuno-oncology, CNS, cardiovascular and metabolic disease, autoimmune disease, inflammation, anti-infective)
- 150+ patents
- Contribution to 15+ clinical programs.

BENEFITS OF CHOOSING EUROFINS DISCOVERY FOR YOUR HIT-TO-LEAD DISCOVERY

- DiscoveryOne™ staff directly available throughout the process, from our scientists and engineers to our project managers – We're never more than a phone call or video conference away from answering your questions, collaborating on solutions, and working with you on the best way to get the data you need. We can work within fee-for-service or FTE models, whichever best suits your needs and project.

- Global capacity and compound management to fit your timelines – Eurofins Discovery integrated compound management logistics ensure that your compounds can be received from anywhere in the world and processed for your HTS with the quality and consistency synonymous with our service promise. Regardless of whether you're looking to run your project in North America, Europe, or Asia, we have the capacity without sacrificing quality or turn-around-time.

- Flexibility for any drug discovery project to meet your expectations – From an assay testing study, safety profiling or iterative projects (e.g. lead optimization) and fully integrated drug discovery solution, our experts accompany you throughout your project providing excellence in project management, from helping you define the most appropriate research strategy to implementing the decided tactics and regular follow-up for the most complex and multidisciplinary projects.

- One integrated point of contact that makes your project workable–With a single drug discovery solutions provider that can alleviate the complications of navigating and managing a complex process, DiscoveryOne's project management gives you instant access to all of Eurofins Discovery's resources, while handling details for you such as:

  - Logistics & billing

  - Experimental data format requirement

  - Scheduling and laboratory coordination across multiple continents

  - Regular reviews allowing you to drive your programs with full transparency and knowledge

  - Access to deep expertise in all phases of discovery and our experience in setting up comprehensive and successful drug candidate screening programs

"Eurofins team members are experts in supporting a complete drug development cycle for new chemical entities and they routinely work with the largest and most innovative biopharmaceutical companies in the world."

–Managing Director of a clinical drug development company in personalized medicine and health care technology services

To learn more about DiscoveryOne IDD solutions, to speak with our experts, and meet the DiscoveryOne IDD experts, visit eurofinsdiscoveryservices.com.