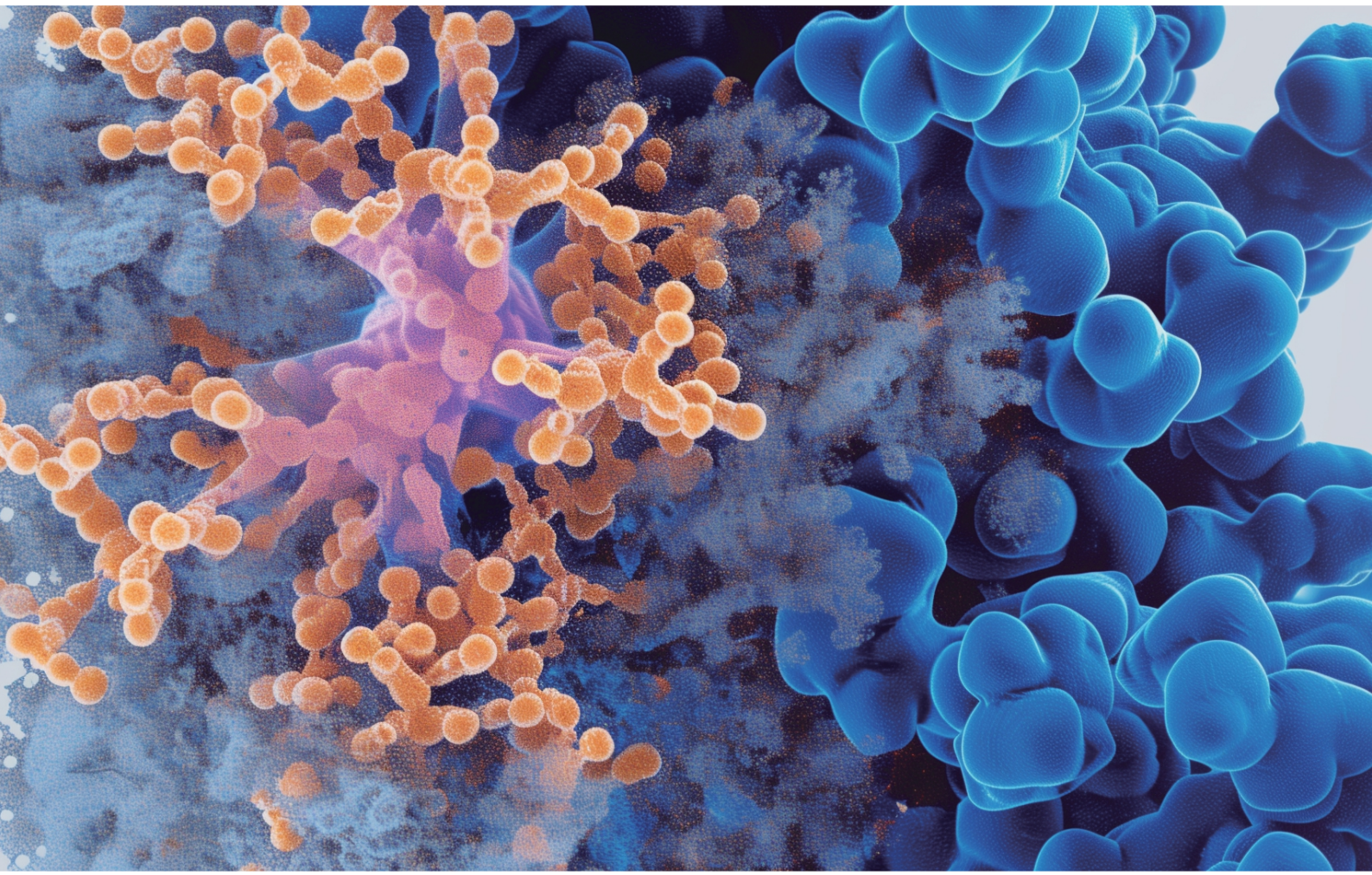
Kinase Integrated Drug Discovery

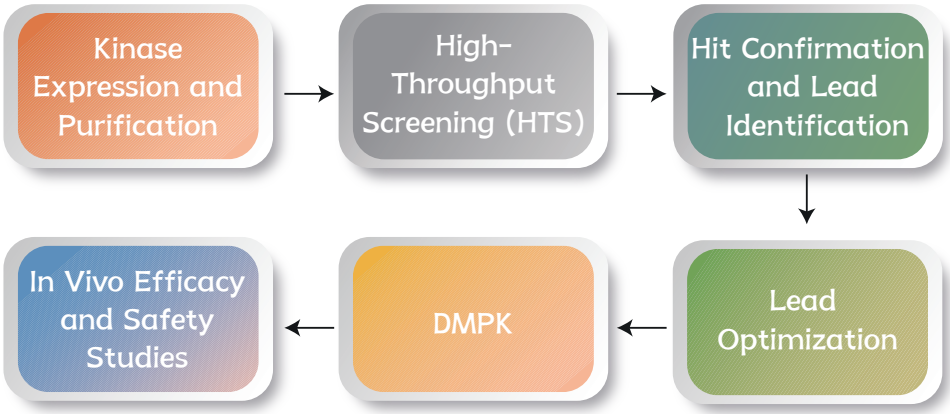
Unlocking Potential through Targeted Solutions



Innovative CRO+Explorer
ICE Bioscience

Advancing Kinase Drug Discovery with ICE Bioscience

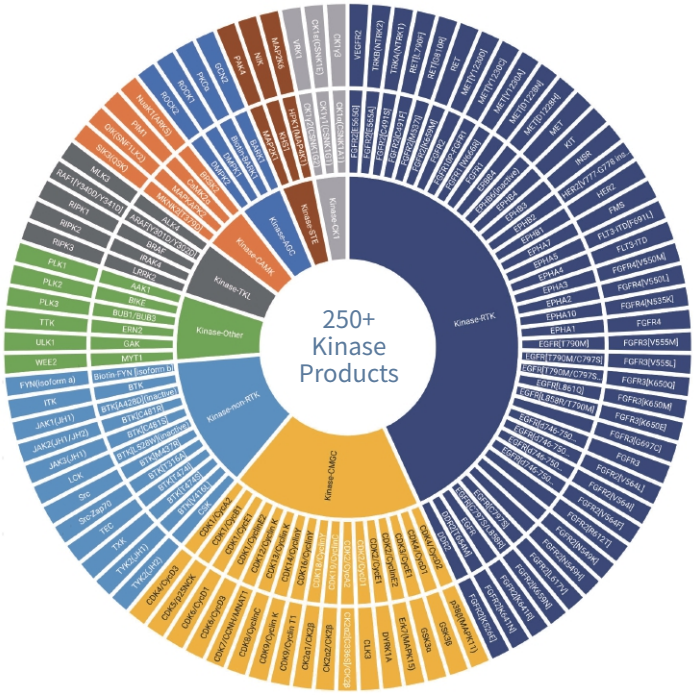
Kinase drug discovery remains a vital area in developing targeted therapies for diseases like cancer, inflammatory disorders, and more. Despite significant advances, challenges persist, including overcoming drug resistance, improving selectivity to minimize off-target effects, identifying novel kinase targets, and tailoring treatments to individual patients through personalized medicine. Additionally, expanding the use of kinase inhibitors to treat non-cancerous diseases opens new avenues for research and development. Addressing these unmet needs requires innovative approaches in drug design, a deeper understanding of disease mechanisms, and the integration of new technologies to refine clinical strategies, promising a future of more effective and precise treatments in kinase-targeted therapy.



Recombinant Proteins

At ICE Bioscience, our kinase protein production is distinguished by our rigorous process that ensures not only the expression and purification of over 250 kinase proteins but also their functional validation.

We also demonstrate advanced capabilities in the co-expression and purification of protein complexes, such as the CDK2 & CycE1 binary complex, which are vital for kinase drug discovery. You could benefit from the availability of these complexes for use in a variety of applications, such as screening for inhibitors, studying protein-protein interactions, and understanding the complex's role in cell cycle regulation and its implications in disease states.



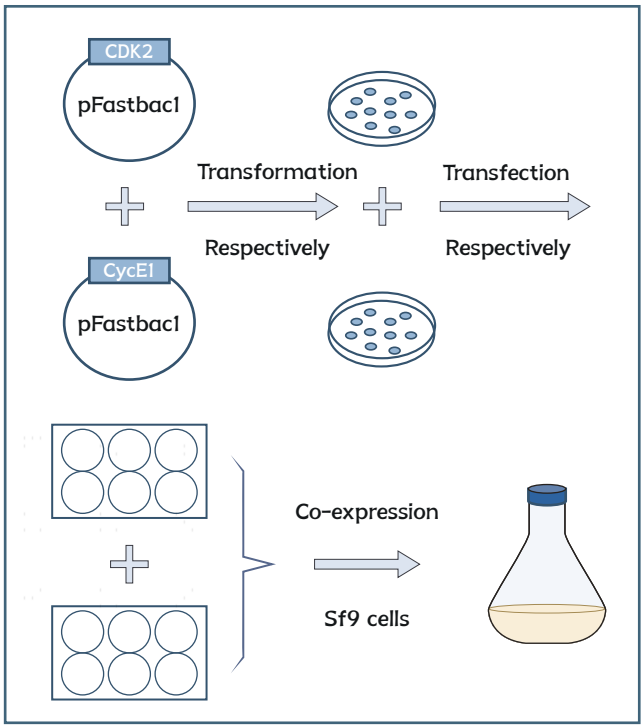


Figure 1. CDK2 & CycE1, forming a binary complex, were co-expressed in SF9 cells.

Kinase Biochemical Assays

ICE Bioscience's extensive portfolio comprises a large collection of kinase assays available, making us a leader in the field of drug discovery. Our expertise extends to developing custom-tailored assays to meet the unique needs of our clients. With a guarantee to provide the specific kinase assay required, researchers and organizations can confidently partner with us to advance their projects. Our expansive portfolio features over **600 kinase assays**, available in a variety of formats to suit diverse research needs and applications:

- **Luminescence-based activity assays (ADP-Glo):** These assays measure kinase activity by detecting the amount of ADP produced, using luminescence for high sensitivity and a broad dynamic range, making them suitable for high-throughput screening.
- **HTRF-based activity assays:** Homogeneous Time-Resolved Fluorescence (HTRF) assays are utilized for their high-throughput capability and robustness, offering a non-radioactive method to measure kinase activity through energy transfer between donor and acceptor molecules.

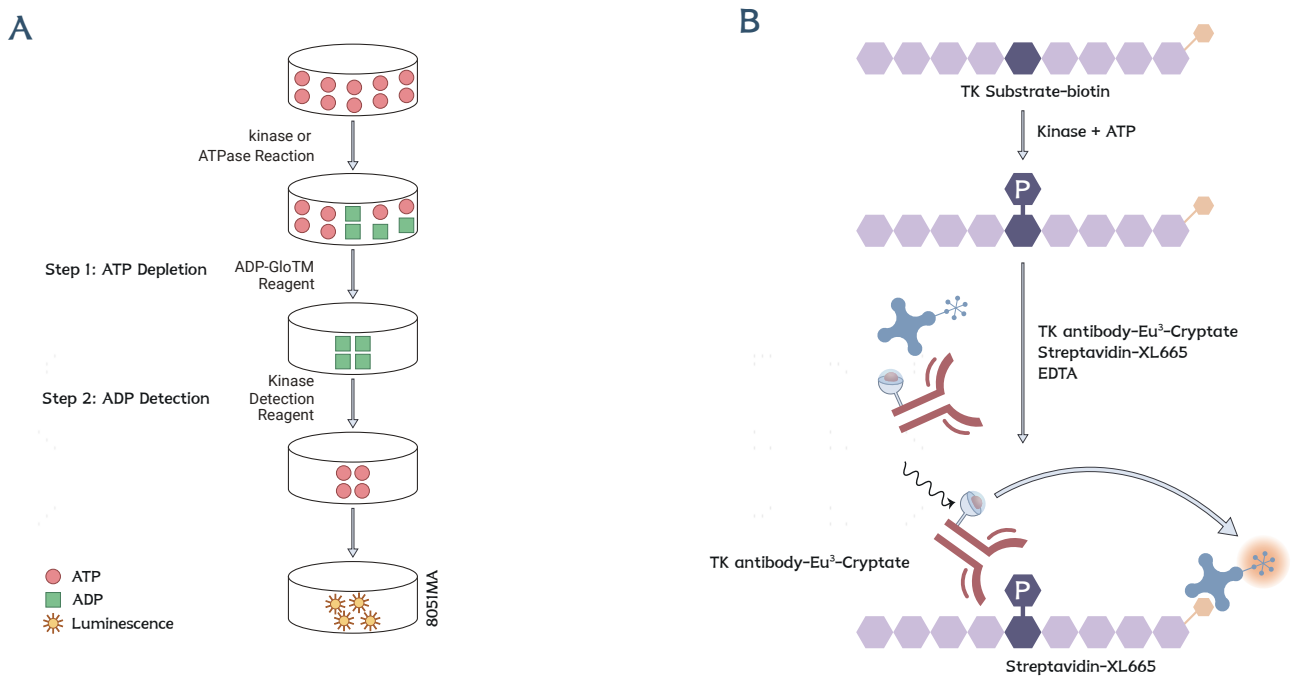


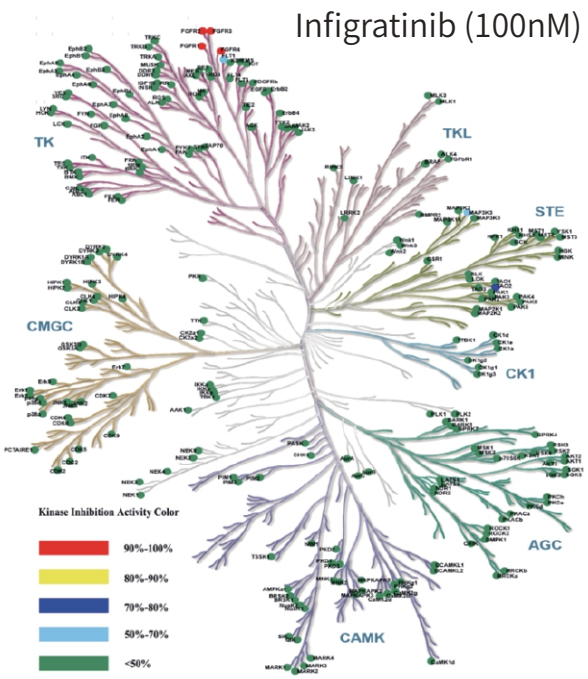
Figure 2. (A) The ADP-Glo™ assay measures kinase activity by quantifying ADP production, with luminescence indicating the enzyme's activity post-reaction. (B) HTRF assays employ donor and acceptor fluorophores to detect phosphorylated substrates, with the resulting fluorescence proportional to kinase activity.

Kinase Panel Screening

Kinase panel screening is a high-throughput method used in the early stages of kinase drug discovery to rapidly assess the activity and specificity of potential therapeutic compounds across a broad spectrum of kinase targets.

Kinase Panels	Panel Size	Features
KinomeMINI WT Panel	80	Economical,rapid screening, essential core WT kinases
KinomeMED WT Panel	217	Intermediate panel offering a wider range of WT kinases
KinomeMAX WT Panel	330	Broad spectrum panel for extensive kinase profiling
KinomeFULL Panel	416	Comprehensive coverage of the entire human kinome
TK WT Panel	76	Targeted selection of the tyrosine kinase family
CDK Panel	16	Specialized for the cyclin-dependent kinase family
LIPID Kinase Panel	25	Focused on phosphatidylinositol kinases
Customized Panel	Custom	Tailored to specific research needs, highly flexible

This phylogenetic tree represents the screening results of Infigratinib at a concentration of 100nM across various kinase families, using ICE Bioscience's kinase panel.
Kinases are categorized into families such as TK, TKL, STE, CMGC, CK1, AGC, and CAMK, and are plotted according to their evolutionary relationships. The degree of inhibition exerted by Infigratinib is color-coded. This visualization allows us to quickly assess the compound's potency and specificity by identifying which kinases are most affected within and across different families, aiding in the determination of potential therapeutic and off-target effects.

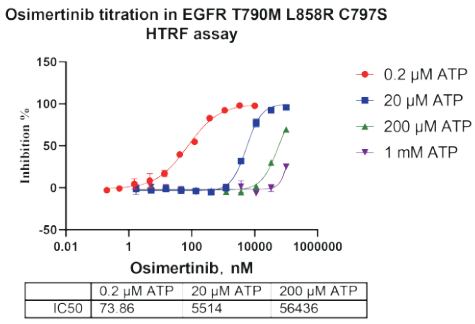


- **High Reproducibility:** Our assays are consistently reliable, ensuring validated results for confident comparisons in diverse research settings.
- **Phylogenetic Visualization:** We provide a phylogenetic tree visualization of screening results for clear insights into compound specificity and kinase family activity.
- **Flexible Deliverables:** Choose from single-point inhibition percentages for a snapshot of activity or detailed IC50 values for in-depth potency analysis.

MoA Study

ATP Competitive Test for Kinase Inhibitor

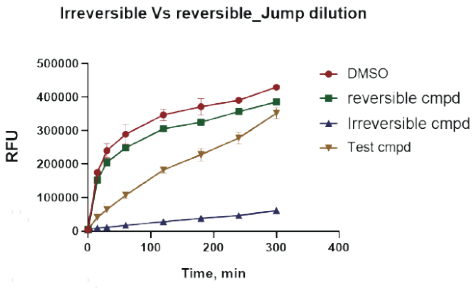
This assay determines if an inhibitor competes with ATP at the kinase's active site, with inhibitor potency decreasing as ATP concentration rises, indicating ATP competition.



	0.2 μM ATP	20 μM ATP	200 μM ATP
IC50	73.86	5514	56436

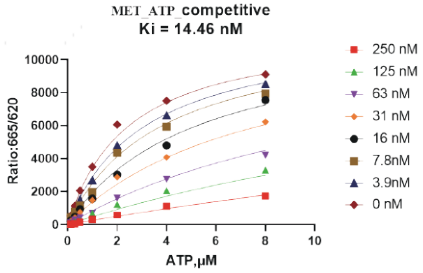
Jump Dilution for Reversible and Irreversible Inhibitor

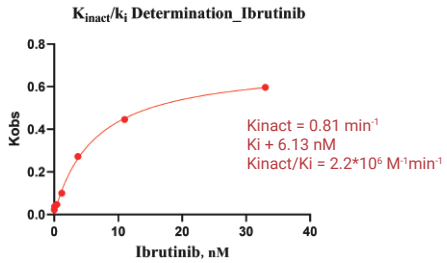
The assays differentiates reversible from irreversible inhibitors by measuring kinase activity after dilution; a reversible inhibitor's effects are diminished, whereas an irreversible inhibitor's effects persist post-dilution.



Inhibition Mode and Ki Determination

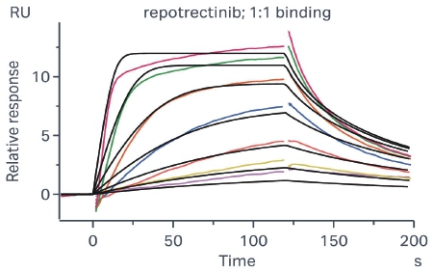
We perform kinetic analysis to determine the inhibition mode and calculate the Ki value, indicating the inhibitor's binding affinity to the kinase.





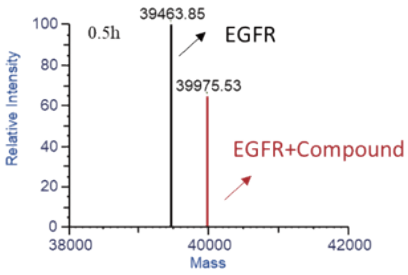
Kinact/ki Test for Covalent Compounds

We assess the reaction rate (kinact) and pre-covalent affinity (Ki) for covalent inhibitors, providing insights into potency and binding permanence.



Biophysical Assays Using SPR

We measure determine binding kinetics (association and dissociation rates) and affinity (KD) of kinase inhibitors. This technique is label-free and provides a direct measurement of the binding event, making it highly valuable for understanding the interaction dynamics between kinases and their inhibitors.

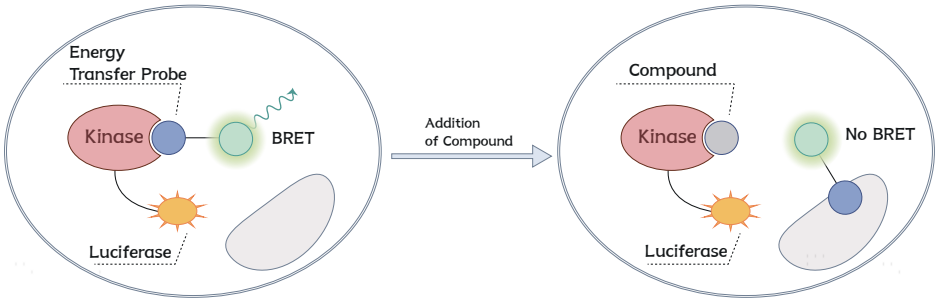


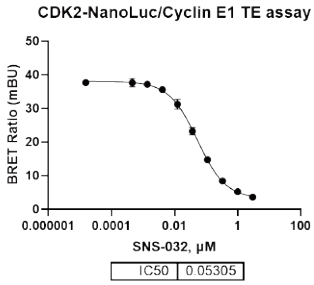
Covalent Assays Using LC-MS

LC-MS is used to identify and characterize covalent bonds formed between a kinase and an inhibitor. By providing mass spectral data before and after the reaction, LC-MS can confirm the formation of a covalent complex and the mechanism of irreversible inhibitors and can also provide the location of binding through mass mapping.

NanoBRET Target Engagement Kinase Assay

ICE Bioscience offers an extensive selection of over 160 NanoBRET TE kinase assays. This assay is a cutting-edge method used for measuring the interaction between a kinase and a test compound inside living cells. It employs Bioluminescence Resonance Energy Transfer (BRET) technology, which is a proximity-based assay where energy transfer occurs between a bioluminescent donor and a fluorescent acceptor when they are close together.



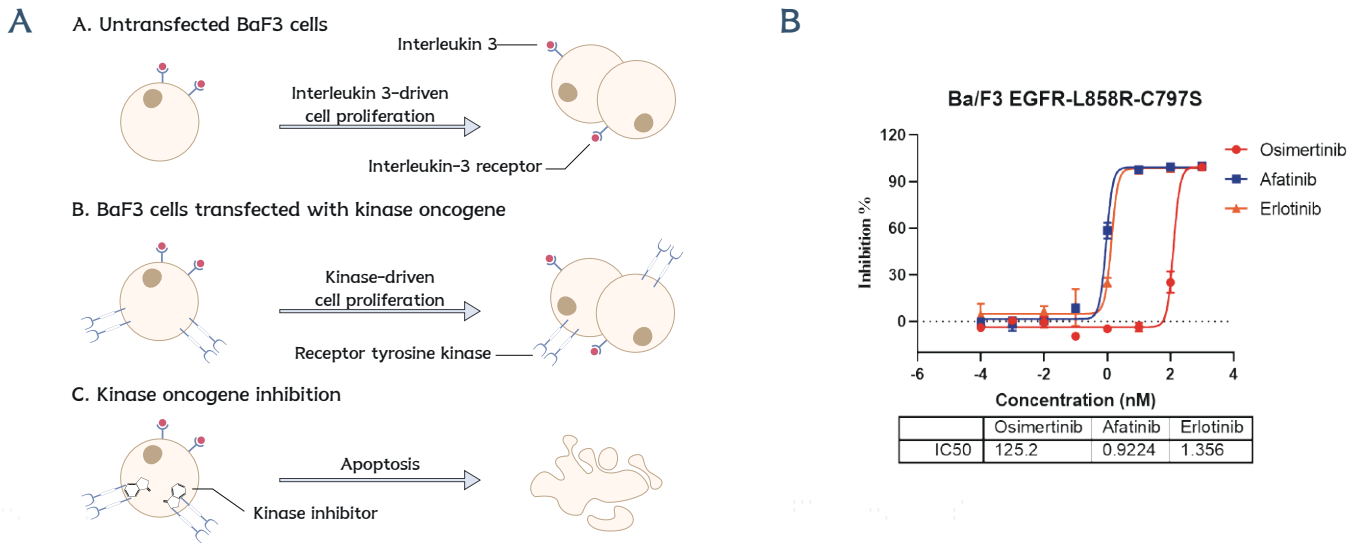


The BRET ratio decreases as the concentration of the inhibitor (SNS-032) increases, indicating the inhibitor's binding to the kinase complex.

- **Live-cell Application:** Unlike traditional assays that require cell lysis, NanoBRET assays are performed in live cells, preserving the natural cellular context and allowing real-time kinetic measurements.
- **Quantitative:** Provides quantitative data on the binding affinity and occupancy of test compounds with kinase targets in intact cells.
- **Throughput Flexibility:** Compatible with both high-throughput screening and detailed mechanistic studies, making it versatile for different stages of drug discovery.

BaF3 Cell Proliferation Assay

The BaF3 Cell Proliferation Assay utilizes the BaF3 cell line, a murine pro-B cell line that depends on interleukin-3 (IL-3) for growth and survival. This assay is commonly used to assess the potency and efficacy of drugs targeting hematopoietic and lymphoid malignancies, where BaF3 cells are engineered to express oncogenic kinases driving cell proliferation. It is useful for the investigation of kinase-engaged signaling pathways and testing of off-target effects of kinase inhibitors.



	Osimertinib	Afatinib	Erlotinib
IC50	125.2	0.9224	1.356

Figure 3. (A) BaF3 cells require IL-3 for proliferation. When testing a compound, the cells are engineered to express a target protein. The overexpression of receptor tyrosine kinases enables the BaF3 cell line to grow without the supplement of IL-3. If the compound effectively inhibits the target, the cells will stop proliferating or die in the absence of IL-3, indicating the compound's potential as a therapeutic agent against cancers driven by that specific kinase activity. (B) The inhibition curves for Ba/F3 cells expressing the EGFR-L858R-C797S mutation, treated with Osimertinib, Afatinib, and Erlotinib, are demonstrating the relative potency of each inhibitor against this specific EGFR mutant.

BaF3 Cell Panel Screening

BaF3 Cell Panel Screening serves as a reliable tool for evaluating drug efficacy and exploring the mechanisms of drug resistance and sensitivity, which is crucial in the development of targeted cancer therapies.

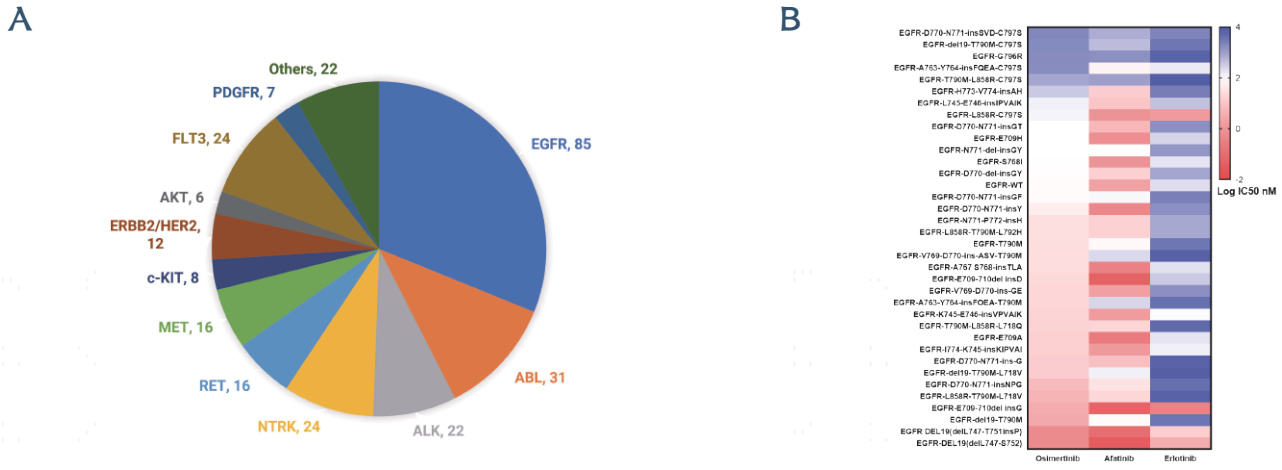


Figure 4. (A) Our comprehensive Ba/F3 cell panel, detailing the number of cell lines available for each kinase target. (B) The results of a BaF3 panel assay, showing the Log IC50 values for various EGFR mutants when treated with different inhibitors: Osimertinib, Afatinib, and Erlotinib. Each row represents a specific EGFR mutation and the color gradient reflects the potency of inhibition, with red indicating higher potency (lower IC50) and blue indicating lower potency (higher IC50).

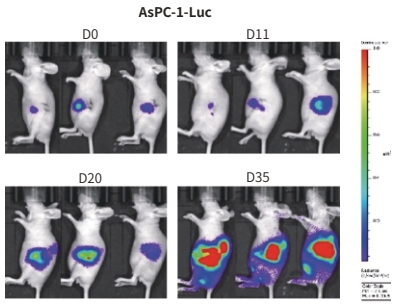
Cell-Based Kinase Assay Portfolio

ICE Bioscience offers a comprehensive suite of cell-based kinase assays, leveraging advanced detection methods and diverse cell models to evaluate kinase activity, inhibition, and protein interactions. Our assays deliver precise, real-time insights into kinase dynamics, signaling pathways, and the mechanisms of action for potential therapeutics. Tailored for both endogenous and exogenous kinase sources, our assays are designed to meet the highest standards of reproducibility and sensitivity for robust drug discovery and development.

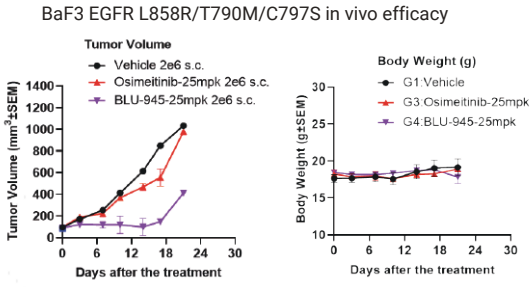
Kinase Panels	Assay Technology	Cell Type	Assay Type	Readout
Cellular Phosphorylation Detection	ICW/IFA, AlphaLISA, HTRF, ELISA, MSD, Flow Cytometry, WB	Adherent or Suspension Cell Line	Substrate Phosphorylation, Protein Degradation	Kinase Inhibition Activity
BaF3 Cell Proliferation	CellTiter-Glo (CTG)	BaF3 Cell Line	Cell Proliferation	Kinase Inhibition Activity
Functional Assay	CellTiter-Glo (CTG), ELISA, AlphaLISA, MSD	Various (Including Tumor Cell Lines)	Cell Viability, Cytokine Release	Kinase Inhibition Activity
Reporter Gene Assay	Luciferase, etc.	Various Cell Lines	Signal Pathway Activation	Transcriptional Activity
NanoBRET™ Target Engagement	NanoBRET™	Various Cell Lines	Protein-Protein Interaction, Kinase Inhibition	Binding Affinity, Compound Potency
HiBiT-Based Protein Degradation Assay	Luminescent Detection	HiBiT Cell Lines	Protein Degradation	Degradation Activity

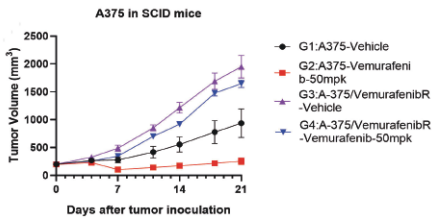
In Vivo Tumor Models

ICE Bioscience specializes in in vivo tumor models, offering a suite of cell lines for oncological research, alongside CDX models and comprehensive pharmacological analyses to streamline the development of kinase-targeted therapies.



Pancreatic Orthotopic tumor model





WT Cell Line Models:

Utilizing wild-type cell lines, these models are instrumental for evaluating the fundamental biology of kinase targets and the primary effects of kinase inhibitors in an in vivo setting. They offer a baseline understanding of drug efficacy and pharmacokinetics in normal biological contexts.

Engineered Ba/F3 Cell Line Models:

These models leverage Ba/F3 cells engineered to express specific kinases or mutations of interest, providing a powerful platform for studying targeted therapies under physiological conditions. They are particularly useful for assessing the potency and specificity of kinase inhibitors against desired targets.

Drug-Resistant Cell Line - CDX Models:

Our CDX (Cell Line Derived Xenograft) models incorporate cell lines with known drug resistance, allowing for the examination of kinase inhibitor efficacy against resistant cancer phenotypes. This approach is critical for the development of second-line therapies and understanding resistance mechanisms.

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.

To learn more, please visit: en.ice-biosci.com