REACTION BIOLOGY

E-BOOK

Mapping the Kinome: Your Guide to Strategic Kinase Panel Screening

Are you confident your kinase screening strategy is optimized for success? Choosing the right assay and kinase panel directly impacts your ability to bring safe, effective therapies to market quickly.

In this comprehensive eBook, you'll gain clear, actionable insights that help you:

- Choose a screening strategy that delivers precise, biologically relevant data
- Identify potent yet selective kinase inhibitors
- Minimize risks associated with off-target interactions
- Accelerate drug discovery timelines while optimizing resources

Let's discover together.

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Kinome Quick Fact

Kinases are one of the largest and most validated classes of "druggable" targets, with more than 85 FDA-approved therapeutic agents that target about two dozen protein kinases.[1]

CHAPTER 1 - Choosing Your Kinase Activity Assay: Methods, Strengths, and Limitations

Choosing Your Kinase Activity Assay: Methods, Strengths, and Limitations

Kinase screening panels set the foundation for the success of your drug discovery program. The wrong choice can lead to misleading data, unnecessary delays, and increased risk of late-stage failure.

Each assay type offers distinct advantages and limitations, and the choice depends on multiple factors. These include the precision and sensitivity required, throughput capacity, available laboratory resources, sample type, and the specific kinase-substrate combination you're evaluating.

Biochemical kinase assays typically measure activity by quantifying the phosphorylation of substrates through radiometric, fluorescence-based, or luminescence-based methods. Alternatively, binding assays can quantify direct interactions between small molecules and kinases, often at the ATP-binding site.

We've put together a quick-reference table with an overview of common kinase assay platforms, including how they work, key strengths, and limitations.

Radiometric activity assays

These assays directly quantify the transfer of a radioactive phosphate group from ATP to a substrate, with reaction mixtures spotted onto filter papers (dot-blot method).

The radioisotope-labeled substrate then binds to the filter, while unreacted phosphate is removed via washing.

- ⊕ Provides a true and precise measure of catalytic activity.
- ⊕ No requirement for modified substrates or coupling enzymes.
- ⊕ Can be applied to any kinase inhibitor, representing a truly universal assay.
- ⊕ High sensitivity and versatility—suitable for both kinase inhibitors and activators.
- ⊖ Careful handling and disposal of radioactive materials is required, making it challenging for research teams to perform in-house.

Scintillation proximity assays (SPA)

Similar to radiometric activity assays, SPA also uses radioisotope-labeled ATP. However, detection occurs via scintillant-coated beads or plates and tagged substrate.

- ⊕ This assay also detects true kinase activity, but indirectly (when the radioisotope is brought into proximity with the beads via binding to the substrate).
- ⊕ No wash step is required for bead-based assays.
- ⊕ Suitable for high-throughput screening.
- ⊖ Requires radioactive waste management.
- ⊖ Substrate requires modification for capture.
- ⊖ Difficult to adapt for use with protein substrates.

Fluorescence-based activity assays

Fluorescence-based activity assays detect kinase activity by measuring changes in fluorescence that occur upon phosphorylation of fluorophore-tagged substrate.

Formats include Fluorescence Resonance Energy Transfer (FRET) and Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET), which use fluorophores with long decay times.

- ⊕ Simple and suitable for high-throughput screening.
- ⊕ High sensitivity and versatility—suitable for both kinase inhibitors and activators.
- ⊖ Relies on synthetic substrate modified for fluorescent labeling, which may interfere with compound/drug binding.
- ⊖ Often relies on antibody or coupling enzyme for detection instead of directly monitoring phosphorylation products.
- ⊖ Can experience higher background signals from certain compounds.

Luminescence-based activity assays

Uses luciferase to measure the amount of ATP.

In the presence of ATP, luciferase converts luciferin to oxyluciferin, resulting in the emission of light.

- ⊕ Offers direct activity measurement.
- ⊕ Suitable for high-throughput screening and can accommodate fluorescent compounds, providing they do not inhibit luciferase.
- ⊖ Luminescence-based assays can exhibit low sensitivity when concentrations of ATP are low.
- ⊖ The coupling enzyme requires counter screening.

Competition binding

Competition binding assays—also known as ligand binding assays—quantify the binding affinity of small molecules to the kinase active site.

Standard probe or tracer-conjugated ligands are measured alongside the test compound, both competing for binding to the kinase active site.

- ⊕ Suitable for non-active form kinases or psedo-kinases.
- ⊖ Performed in the absence of ATP and substrate.
- ⊖ Limited to kinases with known ligands.
- ⊖ Substrate-specific inhibitors are harder to detect.

Mobility shift

These assays take place in microfluidic chips or small plate-based systems, using electroporation to separate phosphorylated and non-phosphorylated substrates based on the difference in charge.

Substrates are labeled with fluorophores, and fluorescence intensity is measured to determine kinase binding.

- ⊕ Suitable for high-throughput screening.
- ⊖ For use with peptide substrates only.
- ⊖ Substrates must be modified for fluorophore labeling.

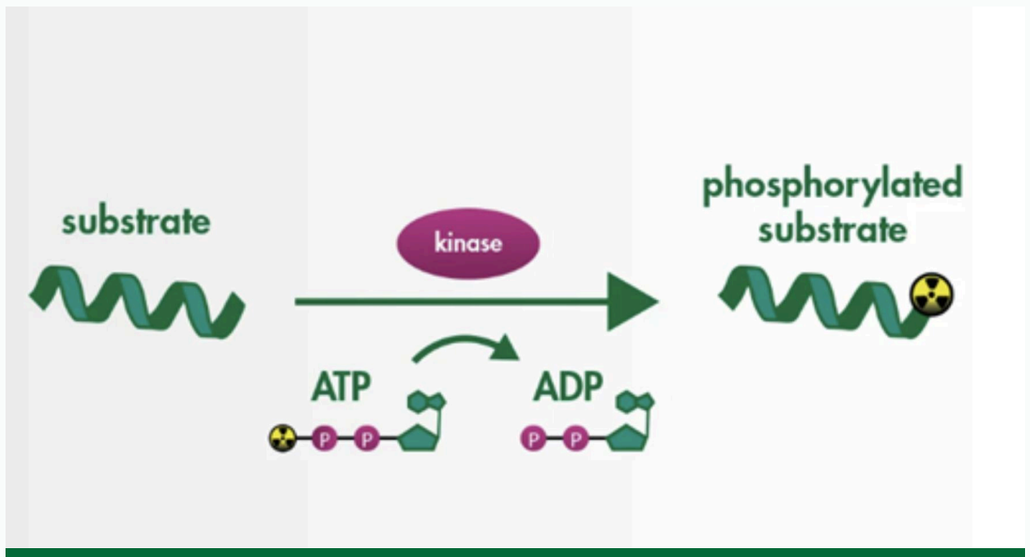
CHAPTER 2 - Radiometric Activity Assays: The Gold Standard

Radiometric Activity Assays: The Gold Standard

Radiometric kinase activity assays are considered the gold standard because they provide direct, highly sensitive, and precise quantification of kinase activity without interference from fluorescent or chemical modifications.[2-5]

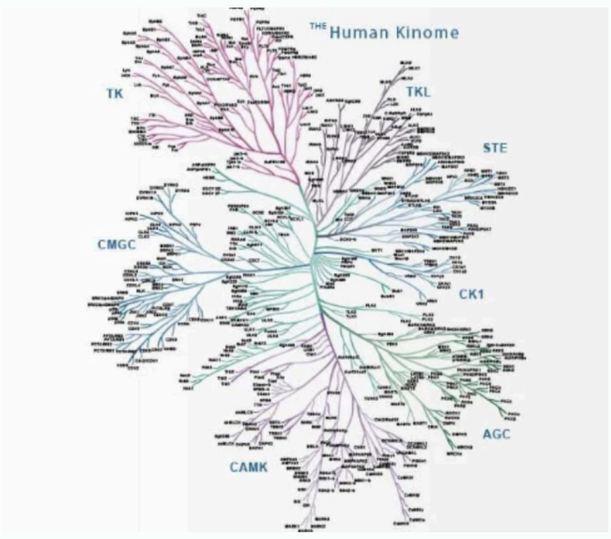
This assay approach offers consistent and robust results with low background signal, and is the only format that directly detects the true product without the use of modified substrates, coupling enzymes, or detection antibodies.

At Reaction Biology, our team brings decades of experience in radiometric activity assays and offers a comprehensive suite of kinase screening services—designed to help you generate high-quality data and reach your research goals with confidence.



Ideal for precise and high-quality data to confirm suspected kinase activity

	Measures kinase activity	Detects all types of inhibitors, including substrate-specific inhibitors	Accommodates both peptide and protein substrates	No modified substrates	Universally applicable to all kinases
Radiometric assay	✓	✓	✓	✓	✓
FRET peptide	✓	✓	✗	✗	✓
Luminescence	✓	✓	✓	✗	✓
Mobility shift	✓	✓	✗	✗	✗
Competition binding (ligand binding and EFC)	✗	suboptimal	✗	✗	✗



HotSpot™ Kinase Screening Service

When accuracy matters most, our HotSpot Kinase Screening service delivers best-in-class radiometric activity assays for precise, high-quality data.

Using a proprietary radiometric filter binding assay, this assay is optimized to maximize sensitivity and minimize false positives and negatives—offering a complete and reliable picture of your compound's kinase activity and selectivity.

Benefits of the HotSpot Kinase Screen include:

1. **The industry's largest human kinome panel**—featuring up to 800+ kinases including specialized panels such as CDK, EGFR, and more.
2. **Available at physiologically relevant 1mM ATP concentrations** along with options for 1µM, 10µM, or an apparent ATP-Km of up to 100µM.
3. **Quickly identify potent, selective kinase inhibitors**—Select ATP-Max Diversify, a tailored panel of representative isoforms from each major kinase family for rapid insights.
4. **For a comprehensive and deep understanding**—Screen your compound with ATP-Max KinomeScreen, a full panel 340 wild type kinases.

Kinome Quick Fact

Imatinib (Gleevec), a tyrosine kinase inhibitor, was approved by the FDA in 2001 after only 72 days of review. Imatinib set the foundation for the rise of targeted therapy and remains a shining example of rational drug design.[6]

Radiometric Assay Formats: HotSpot vs. ^{33}PanQinase™

Reaction Biology offers two radiometric activity assay formats that are performed in our screening facilities on a monthly or bi-weekly basis. Protein kinases are screened via HotSpot assay in Malvern, PA, USA, and via ^{33}PanQinase assay in Freiburg, Germany.

Both assays are based on the transfer of ^{33}P-labelled phosphate from ATP to the kinase substrate. The methods, however, differ slightly with respect to retention and detection of the phosphorylated substrates.

Is HotSpot or ^{33}PanQinase right for your project?

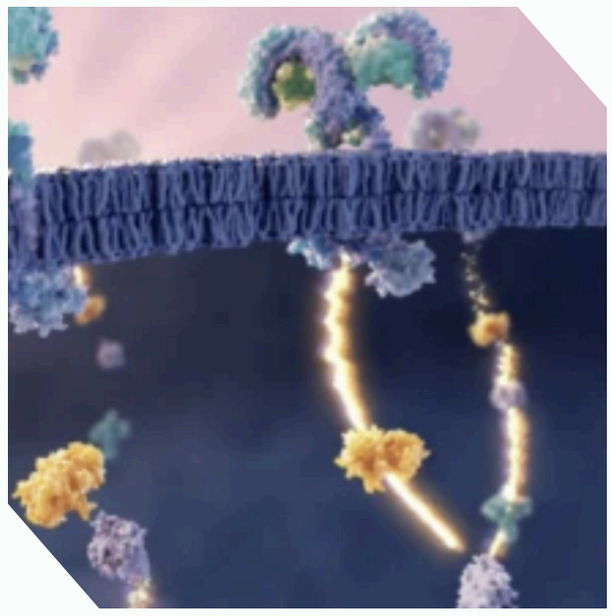
	Malvern, PA, USA HOTSPOT	Freiburg, Germany ^{33}PanQinase
Retention and detection of substrates	Captured via spotting of the reaction mix on a filter membrane; free ATP is washed off	Performed with ScintiPlate microtiter plates, which are coated with scintillant for detection
ATP concentrations	1mM, 1µM, 10µM, or apparent ATP-Km up to 100µM	Apparent ATP-Km
Timelines	Selectivity panel screening is performed twice per month. Reports are sent 2 to 3 weeks after the project start date.	Selectivity panel screening is performed once per month or on-demand. Reports are sent 10 business days after the project start date.
Screening schedule	Find upcoming screening dates here.	Find upcoming screening dates here.

Learn more about the panels offered at each facility by visiting our webpage, or contact our team for assistance.

"Reaction Biology Europe has provided their service for numerous projects with us, especially on multiple kinase assays… which have guided our way through drug development. I was very impressed not only by their level of expertise and professionalism but also by their enthusiasm and drive to deliver without delay. The insightful advice they provide has been instrumental to our successes, and the reproducibility, quality, and reporting of the results have been outstanding throughout the many years we have been working together."

Dr. Laurent Meijer
Chair & Chief Scientific Officer
Perha Pharmaceuticals & ManRos Therapeutics

ADP-Glo™ Kinase Assays



When you're looking for a rapid solution for primary screening and early profiling of kinase selectivity, the ADP-Glo Kinase Assay can help to quickly identify potential hits and accelerate your research timelines.

This approach is ideal for validating and expanding on internal test results, and allows for a seamless transition to a deeper interrogation with radiometric assays.

Benefits of ADP-Glo Kinase Assays include:

1

An expanding panel of ADP-Glo kinase targets—including more than 200 targets.

2

Activity detection for a range of kinases—screen a wide range of kinases, including low-activity kinases. Standard screens are run at the respective Km [ATP] for each kinase to ensure biochemically balanced assay conditions.

3

Scalable—our service is customizable to meet the demands of your project, from small-scale introductory screens to large-scale kinase profiling.

4

Comprehensive data analysis—receive detailed and comprehensive data analysis, enabling you to make informed and faster decisions.

Custom Kinase Inhibition Studies

Standard panels don't always capture the specific kinase families or variants most relevant to your target. Reaction Biology's Custom Kinase Assay Service allows you to design screening strategies or build customized profiling panels that align perfectly with your research objectives.

Mechanism of Action (MOA) Studies

Whether you need to focus on inhibition kinetics, ATP competition, or a reversibility study, our custom kinase assays service lets you streamline your analysis while delivering comprehensive coverage of your areas of interest. This targeted approach can significantly accelerate your timeline by focusing resources on the kinases and substrates that matter most to your research goals.

Recombinant Kinases and Substrates for In-House Assay Development

For assay development in your own lab, purified recombinant kinases and substrates can be purchased directly from Reaction Biology. We offer aliquots off-the-shelf, bulk quantities, or custom-tailored recombinant kinase protein production can be performed according to your specific requirements.

Kinome Quick Fact

Reaction Biology collaborated with scientists at the Fox Chase Cancer Center to publish a landmark study on the kinome in Nature Biotechnology, which has been cited more than 800 times!

CHAPTER 3 - When to Use a Large Panel or Focused Panel

When to Use a Large Panel or Focused Panel

Kinase screening is a crucial step in the drug discovery process for identifying selective and potent kinase inhibitors. Screening can use broad or focused panels, depending on the specific goals and stage of development.

Comprehensive Screening: Avoid Late-Stage Failures

Determining the selectivity of a novel kinase inhibitor by screening a large part of the kinome during the discovery process can reduce the risk of adverse side effects or the inhibition of non-kinase targets.

Comprehensive screening is particularly valuable when:

- Used to screen all early stage compounds to filter out non-selective compounds, allowing you to focus resources on the most selective compounds for lead optimization.
- Investigating a novel compound, mechanism or new therapeutic area—helping to reveal unexpected selectivity patterns before clinical trials.
- Seeking a deep understanding of the full pharmacological profile of your lead compounds, and preparing for regulatory submissions.

Strategic Use of Focused Panels: Cost-Effective Targeting of a Specific Pathway

Focused panels can either include a tailored panel of representative isoforms from each major kinase family—such as the HotSpot ATP-Max Diversify panel, or specialty panels, comprising a selection of kinase-specific mutants, including the tyrosine kinase panel, EGFR mutant profiler and CDK profiler.

Focused screening is particularly valuable when:

- Looking for a more cost-effective and quicker understanding of your compound.
- Optimizing a lead compound and studying its selectivity for kinases relevant to a specific disease.
- You have a specific hypothesis to test or wish to validate internal screening results suggesting activity within a specific kinase family—a targeted panel can map the full selectivity within that family.
- Seeking compounds that inhibit multiple allelic variants of a disease-associated target.

CHAPTER 4 - Kinase-Substrate Optimization: Finding the Right Match Matters

Kinase-Substrate Optimization: Finding the Right Match Matters

Identifying a suitable substrate for a kinase profiling assay can be challenging. The physiological substrate for a given kinase may be unknown, or does not work under in vitro conditions in a screening assay.
However, selecting the right substrate—or a suboptimal one—can affect assay performance and the biological relevance of your screening results.
Optimized substrates can improve assay signal-to-background ratios, sensitivity and specificity, and reduce non-specific interactions. But identifying a new kinase's endogenous physiological substrate and function can be costly and time consuming.

Kinase SubstrateFinder Assay Service

Kinase SubstrateFinder is a biochemical kinase assay that can be used for the identification of suitable substrates for a specific kinase of interest.

The service offers 5 variants of standardized service packages for protein or peptide substrate identification:

- Three generic Kinase SubstrateFinder variants, comprising different panels of proteins used as kinase substrate at Reaction Biology and using the ADP-Glo assay platform.
- Two physiological SubstrateFinder variants, including biotinylated peptide libraries from JPT Peptide Technologies and using the radiometric ^{33}PanQinase assay.

Screening uses the radiometric ^{33}PanQinase assay, with reports available within 10 business days for standard projects, or expedited scheduling and data delivery can be arranged prior to commencement of the studies.

Streamline Kinase Identification with KinaseFinder

If you're exploring a target that seems "undruggable", have phenotypic screening hits where the mechanism is unclear, or are mapping signaling pathways, we can help you streamline kinase identification.

KinaseFinder is our standardized biochemical assay service package for identifying protein kinases which accept a given substrate for phosphorylation.

KinaseFinder can screen your substrate against up to 380 kinases using either the HotSpot or ^{33}PanQinase platforms, providing comprehensive coverage of potential interactions with your peptide or protein substrate.

Step 1

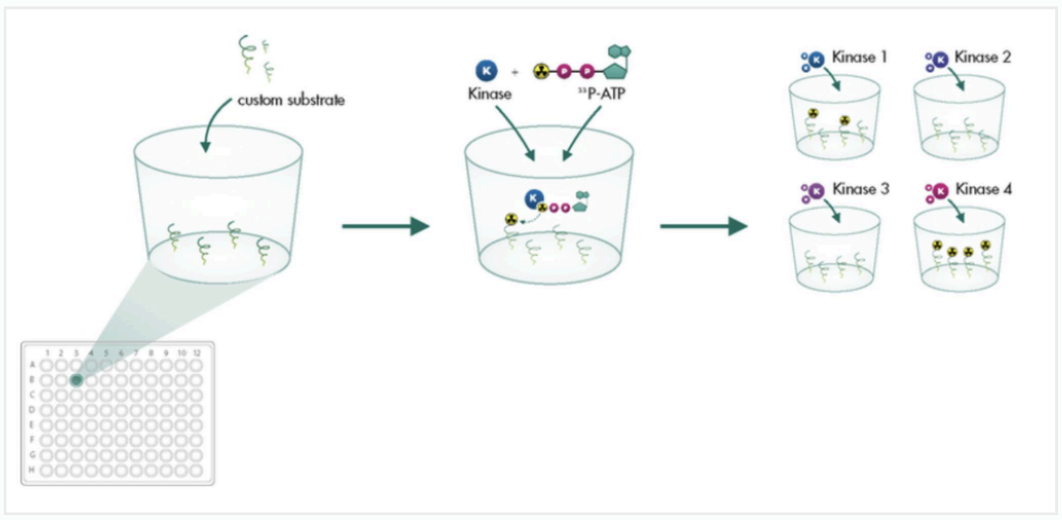
The target substrate is incubated with different kinases in every well of a multi-well plate and ^{33}P-ATP that serves as a phosphate donor.

Step 2

The kinase catalyzes the transfer of ^{33}P when the protein or peptide is recognized as a substrate.

Step 3

Substrates are either bound to scintillant-coated ScintiPlate (^{33}PanQinase) or dropped on filter paper for readout (HotSpot).



Kinome Quick Fact

Reaction Biology's kinase assays were repeatedly ranked #1 in the Kinase Profiling Trends Survey by HTStec, reflecting our industry-leading quality and customer focus.

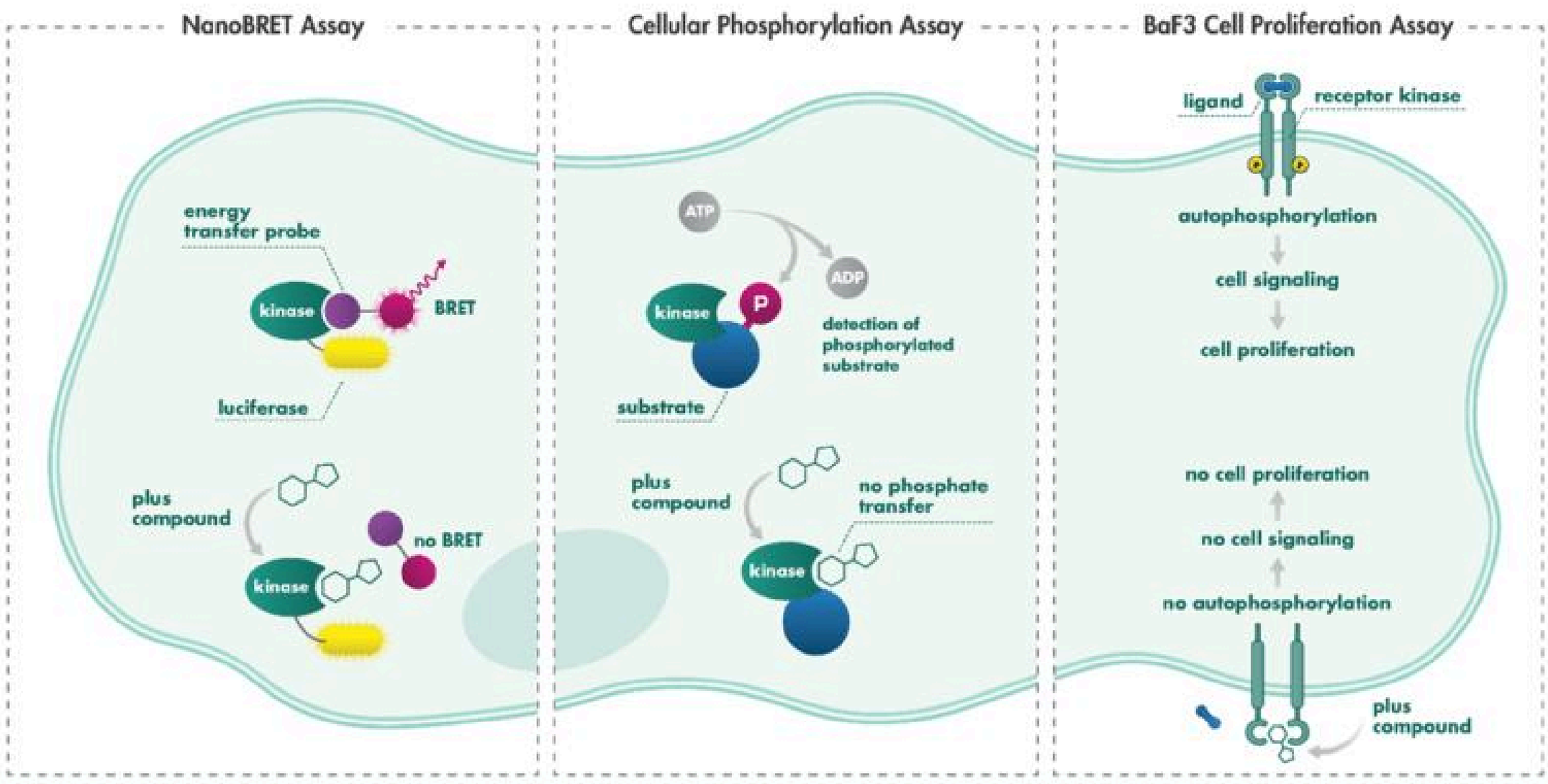
CHAPTER 5 - Cell-Based Assays: An Integrated Workflow for Target Engagement

Cell-Based Assays: Move Forward with an Integrated Target Engagement Workflow

After establishing activity, potency, and selectivity of your kinase inhibitor via biochemical means, the next step is ensure that your compound is effectively inhibiting the kinase within a biological context.

Learn how your drug interacts with its target and affects signalling pathways inside a living cell with our cell-based target engagement and phosphorylation assay portfolio.

Evaluate the selectivity of your kinase inhibitor with advanced functional assays



NanoBRET Assay

Measure the apparent affinity of your test compound(s) for its target within intact living cells.

Cellular Phosphorylation Assay

Evaluate the impact of your test compound on downstream phosphorylation targets.

BaF3 Cell Proliferation Assay

Investigate your kinase inhibitor activity in a controlled setting.

“I have worked with [Reaction Biology] for more than 5 years on several interesting projects. These have ranged from routine enzyme screening to more complex kinome-scan and in-cell target engagement work. The results of these endeavors have led to a series of top-tier publications. This coupled with exceptional customer service means I have no hesitation to recommend Reaction Biology Corp. and Reaction Biology Europe as research partners.”

Dr. Laurent Meijer
Chair & Chief Scientific Officer
Perha Pharmaceuticals & ManRos Therapeutics

We support every step of your kinase drug development

Reaction Biology's Kinase Drug Discovery offering:

- Gold-standard radiometric activity assays established against 800+ kinase targets.
- Three cellular assay formats: i. Phosphorylation assays, ii. Target engagement assays, iii. Kinase-dependent transformation assays.
- Biophysical characterization of the compound-target interaction.
- Recombinant kinase enzymes and substrates for sale.
- Integrated drug discovery for kinase targets.
- Extensive coverage of EGFR and CDK targets.

Ready To Get Started?

Visit our webpage to learn more about how Reaction Biology's comprehensive biochemical, in vitro, and in vivo services can support every step of your kinase drug development pipeline and accelerate your path to success.

Power your pipeline with Integrated Kinase Discovery Services

[1] https://brimr.org/protein-kinase-inhibitors

[2] https://www.sciencedirect.com/science/article/abs/pii/S1740674915000505

[3] https://link.springer.com/protocol/10.1007/978-1-4939-3073-9_7

[4] https://www.reactionbiology.com/sites/default/files/Downloads/files%20from%20old%20website/kinase_assay_profiling_expert_opinion2008.pdf

[5] https://pubmed.ncbi.nlm.nih.gov/26723886/

[6] https://www.cancer.gov/research/progress/discovery/gleevec

[7] https://pmc.ncbi.nlm.nih.gov/articles/PMC3230241/

The premier provider of kinase screening provides scientists worldwide with a trusted research partner supporting all phases of preclinical drug discovery either by integrated drug discovery or fee-for-service. From labs in the USA and Germany, Reaction Biology performs research projects for customers with collaborative support and quality data for a superior research result. We would be delighted to support your research too.

Let's discover together.