BellBrook Labs

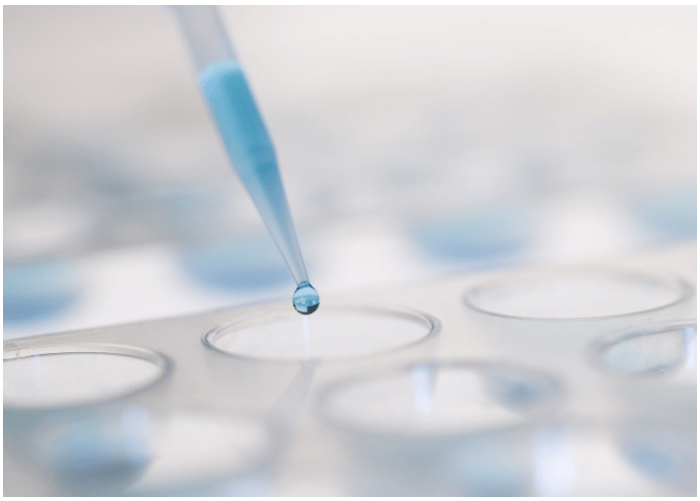
BIOLOGY AT WORK

# Assay Development & Lead Discovery Services

## Drug Discovery Services

# Drug Discovery Services

- **Assay Development**
- **High Throughput Screening**
- **Hit-to-Lead**
- **Lead Discovery**



**BellBrook Labs provides a variety of drug discovery services, including biochemical assay development and lead discovery to advance your target into a lead candidate faster and with confidence.**

BellBrook's drug discovery services specialize in small molecule inhibitors/activators for enzyme drug targets. We have been conducting assay development and lead discovery for over 15 years. During this time, our experts have worked diligently with a variety of customers in their early-stage drug discovery programs. From assay development to hit-to-lead, we can provide quality data to help accelerate your discovery program.

# Accelerating Drug Discovery

As the drug discovery landscape continues to evolve, pressure to improve the efficiency of drug discovery programs has led to a greater demand for characterization and prioritization of lead molecules.

Technologies such as virtual screening have become the latest vehicle moving drug discovery down its arduous path. To verify potential hits from a virtual screen, biochemical assays can be used to provide a fast, effective approach to qualify leads earlier, saving time and resources by eliminating unnecessary studies downstream.

"Some CROs reserve their best teams for high profile customers. If you're not right there with them you get less experienced teams… BellBrook understands enzyme kinetics, so it made that part of the project so much easier. The larger CRO didn't seem to have the expertise we needed in this area."

- BioPharma Lead Discovery Program Manager

There are plenty of CROs in drug discovery, but finding a CRO with experience in enzymology and a portfolio of assays ready for novel targets can be challenging. Over the years, BellBrook's expertise in building the Transcreener platform has helped numerous researchers in drug discovery.

# Assay Development Services

**Whether you want to screen 2M compounds or perform SAR on 20, you need a robust, reliable biochemical assay that has been optimized for your target.**

Leveraging BellBrook's assay development services expertise will save you time during your discovery campaign. BellBrook scientists have optimized Transcreener assays for more than 100 different enzyme targets, including kinases, ATPase, GTPases, glycosyltransferases, ligases/synthetases, and phosphodiesterases. We will define the exact conditions needed for initial velocity detection of your enzyme with outstanding Z' values (0.7 to 0.9 are typical). We will tailor our efforts to meet your specific needs, for example, minimizing consumption of limited reagents or enabling simultaneous screening for inhibitors and activators. And we will work fast. If your target enzyme is in good shape, we can generally complete the assay development services within two to four weeks.

## Services Include:

### Target Protein Sourcing/Production

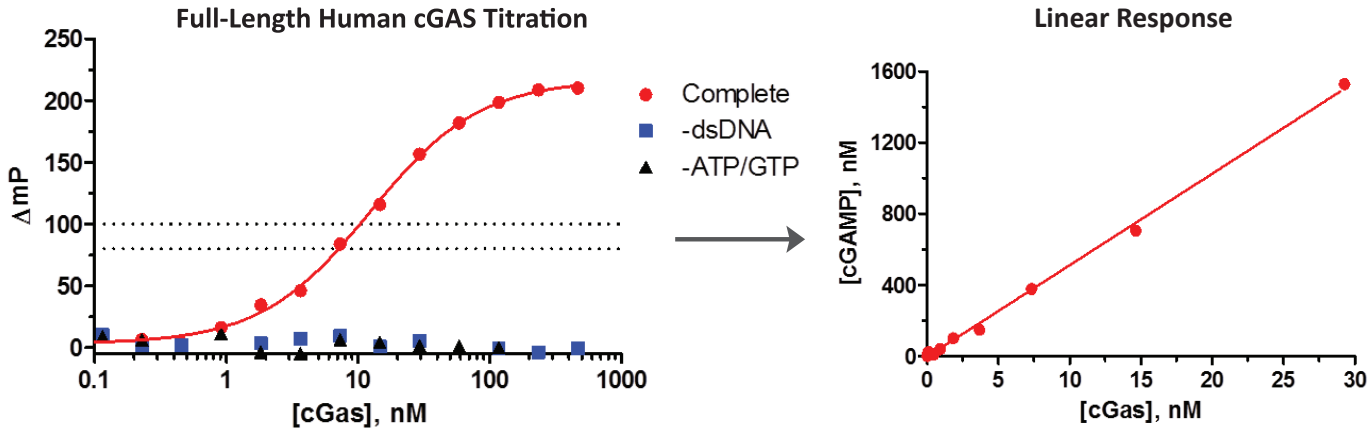
If the target protein is available from the customer or vendor, we will test it for purity and functional activity. If not, we will work with a third-party partner to produce it using the most efficient and cost-effective expression and purification strategy.

### Assay Optimization with Target Protein

We will determine the optimal buffer composition, enzyme concentration, and reaction time and optimize detection reagents to produce the maximal signal under initial velocity conditions.

### Assay Validation

Depending on customer needs, validation could include dose-responses with control inhibitors, determination of kinetic parameters, pilot screens with targeted libraries, or interference screens.




Detection of purified, full-length human cGAS with the Transcreener cGAMP FP Assay. Linear response is shown from polarization data converted to cGAMP using a standard curve.

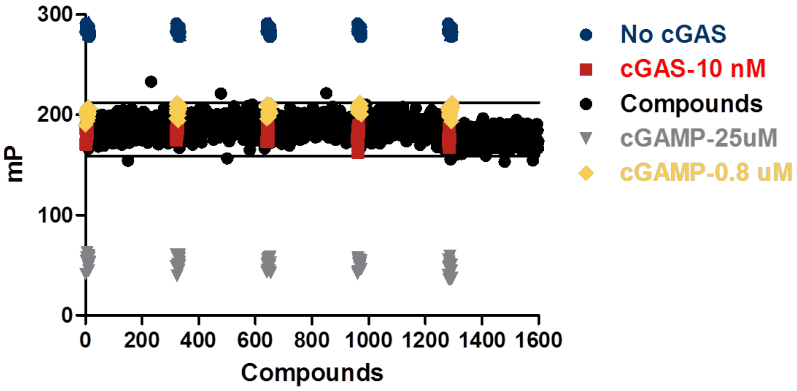
# Lead Discovery Services

**BellBrook scientists will use their extensive biochemistry and enzymology expertise to identify actives from your virtual screen, prioritize your HTS hits, or generate quantitative data to drive your SAR/medicinal chemistry efforts.**

## Services Include:

### Inhibitor Screening

Identify or confirm activity with the target. We can perform single-dose screens as well as multi-dose screening. Screening size can be with our library or yours and range from 100 to 100,000 compounds.




Example screen with 1600 compounds. cGAS was used at 10 nM, compounds were at 10 µM. Z = 0.62, Z′ = 0.7 illustrate a robust screening assay.

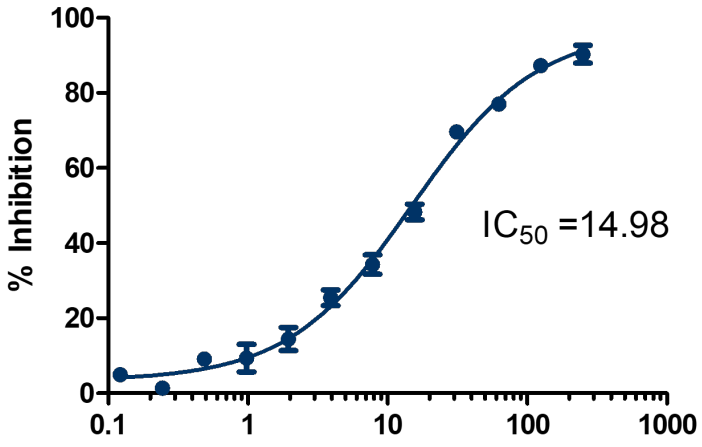
Hits were determined to be anything outside three standard deviations from the mean. Hits can then be assayed in dose-response mode for hit confirmation and follow-up SAR.

### Inhibitor Potency Profiling

Dose-response with target and/or related proteins. We run dose-response assays quickly with the validated Transcreener suite of assays.

Confirmation of a screening hit using the Transcreener cGAMP cGAS Assay in the FP format.

Follow-up assays to triage screening hits can be used to selective bonafide inhibitors for advancement into medicinal chemistry/SAR.



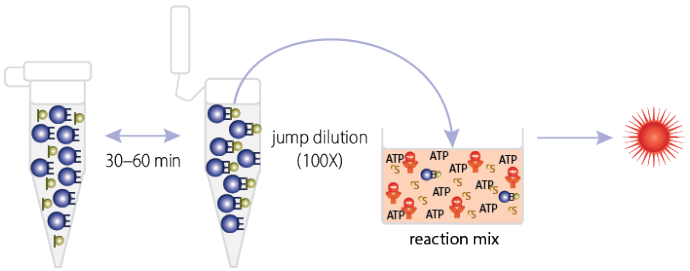

### Inhibitor Selectivity Profiling

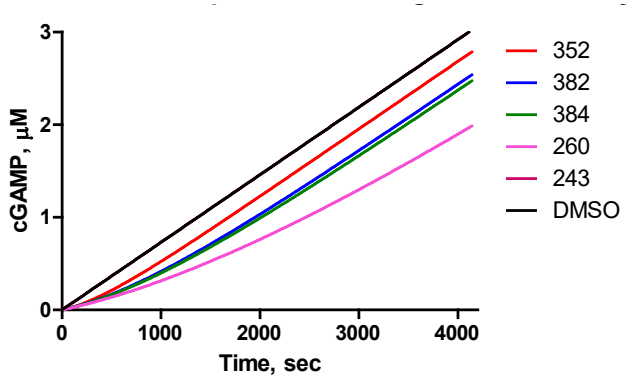
Better understand off-target effects of your compound. Choose from a variety of assays and validated targets. Specialty areas include innate immunity, as well as ATPase and GTPase profiling.

# Lead Discovery Services

## Residence Time Measurements

During drug development initiatives, analysis of drug-target residence times can improve efficacy, increase therapeutic window, and reduce the risk of premature focus on candidate compounds that are likely to have undesirable side effects.

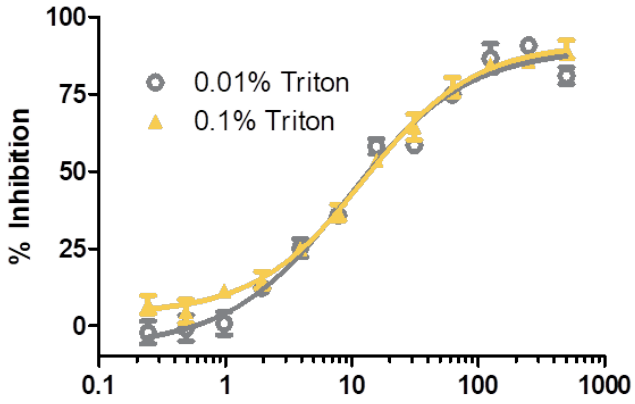


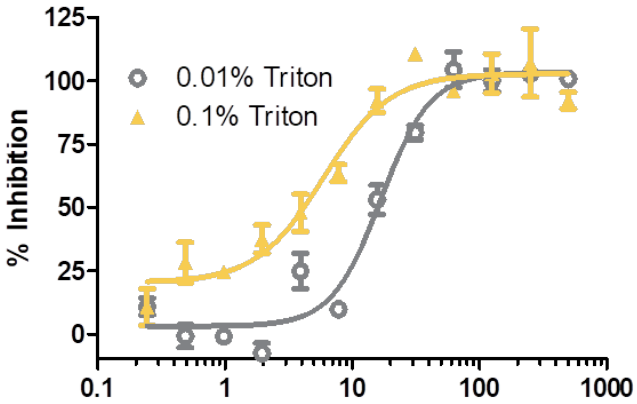




Determination of $k_{off}$ using 'jump dilution' enzymatic assay method. Inhibitors are preincubated with enzyme at saturating concentration to allow formation of E-I complexes, then diluted 100-fold into the reaction mix. Recovery of enzyme activity correlates with inhibitor dissociation. Activity recovers as the E-I complex dissociates, allowing calculation of off-rates.

## Triaging Non-Stoichiometric Inhibitors

Weed-out non-stoichiometric inhibitors by performing assays under different conditions such as varying detergent and enzyme concentration. Reduce wasted time on compounds you can't move forward by eliminating them earlier.




Identification of non-stoichiometric inhibitors (NSI) using inhibitor titration and detergent disaggregation Non-ionic detergent can disperse aggregates, resulting in decreased $IC_{50}$ (above). Excess enzyme titrates out NSIs resulting in a significant increase in $IC_{50}$ (not shown).

## Mechanism of Action Studies

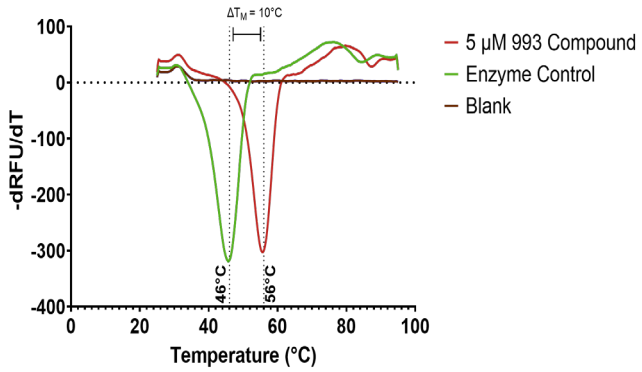
Kinetic analysis to define mode of inhibition. We work to characterize inhibitor MOA during hit-to-lead using substrate competition assays. Determine quickly if your inhibitor is substrate competitive, noncompetitive, or uncompetitive.

# Lead Discovery Services

## Thermal Shift Assay Measurements

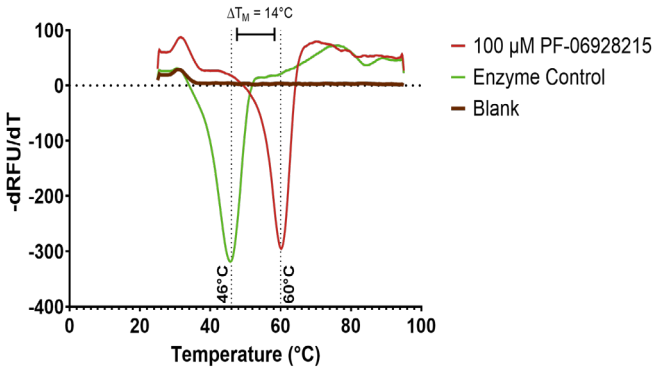
Thermal shift assays confirm a compound's engagement with a target protein by evaluating shifts in protein melting temperature. Our TSA services help characterize protein stability and optimal buffer conditions, determine melting temperature ($T_M$) of a protein, and evaluate compound-protein binding. Avoid the hassle of running an assay in-house with our quick and accurate TSA services.

### cGAS Thermal Shift (993 Compound)




$\Delta T_M = 10°C$

### cGAS Thermal Shift (PF-06928215 Compound)




$\Delta T_M = 14°C$

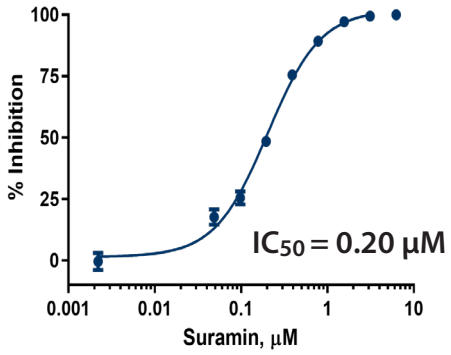
Confirmation of compound-protein binding using the thermal shift assay. Optimal assay conditions are evaluated first to determine the lowest enzyme concentration that produces a robust signal. Thermal shift assays are run with a candidate compound and control. Shifts in protein melting temperature from control to candidate compound confirm compound-protein binding.

## Profiling Panels

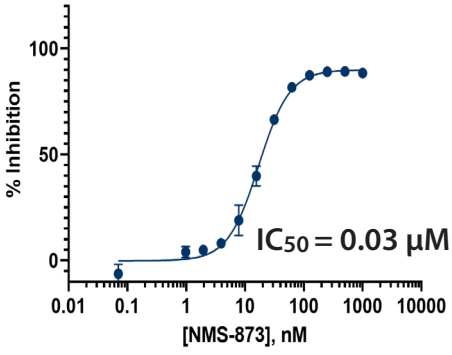
Profiling panels are available for ATPase-like proteins, helicase proteins, cGAS/STING-related proteins, and GTPase proteins. A standard profiling panel is available, and we can customize the panel as needed to meet your needs. If you don't see your target listed, we may still have experience with that target or a similar one within an enzyme family.

| ATPase-Like Proteins | GTPase Proteins | Helicase Proteins | cGAS/STING-Related |
|---|---|---|---|
| p97 | KRAS | DDX3 | cGAS |
| CD39 | HRAS | DDX17 | ENPP1 |
| CD73 | NRAS | WRN | TREX1 |
| KHK | RRAS | POLQ | IKKβ |
| ADK | RhoA | NSP13 | TBK1 |
| DDX3 | RhoC | RIG-I | OAS1 |
| POLQ | Cdc42 | MDA5 | |
| WRN | Rac1 | | |
| NUDT5 | Ran | | |
| ENPP1 | | | |

### ENPP1 Dose Response




### WRN Dose Response



# Lead Discovery Services

## How Does It Work?

### DISCUSS YOUR TARGET

We may have experience with your target or a similar one within an enzyme family. Take a look at some examples at bellbrooklabs.com. We will keep things discrete, confidential, and professional.

### TALK TO A SCIENTIST

Let's talk science. We want to learn more about your target, your goals, and how we can accelerate your lead discovery. We are flexible with differing substrate concentrations (e.g., ATP for kinases) and assay conditions.

### WE'LL DO THE WORK

Send us your compounds. We'll use proven Transcreener Assays to measure enzymatic activity. These measurements can be used to determine $IC_{50}$ values, measure residence time, mechanism of action studies, triage non-stoichiometric inhibitors, and more. Assays will be performed at our lab in Madison, WI.

### QUICKLY PROVIDE A REPORT AND SUPPORT

We'll provide a full report including the raw data and results of the experiments. We can keep things as simple or complex as you like.

# Biochemical Screening Services for Enzymes Based On Proven Transcreener Technology

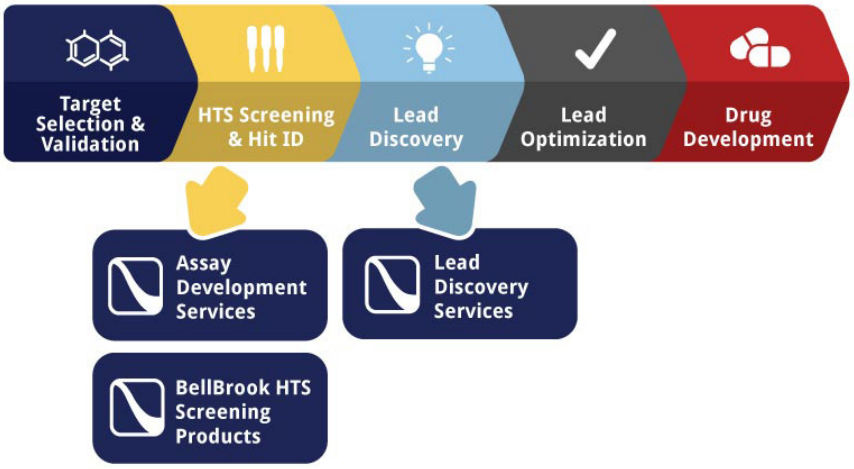
## Example Target Families

- Protein Kinases
- Lipid Kinases
- Carbohydrate Kinases
- ATPases
- Methyltransferases
- HDACs
- GTPases,GAPs,GEFs
- Phosphodiesterases
- Helicases
- Ligases/Synthetases
- Glycosyltransferases

## Example Targets

| | | |
|---|---|---|
| AMPK | cGAS | MLL4 |
| JAK1 | ENPP1 | NSP14 |
| JAK3 | RIG-I | PRMT4 |
| TBK1 | MDA5 | PRMT5 |
| IKK-β | OAS1 | METTL3 |
| ADK | TREX1 | KRAS |
| IRAK4 | DDX3 | HRAS |
| MAPK8 | NSP13 | NRAS |
| PKR | POLQ | RRAS |
| RIPK1 | WRN | RhoA |
| RIPK2 | p97 | RhoC |
| CD73 | KHK | Cdc42 |
| CD38 | PARG | Rac1 |
| CD39 | GALNT2 | Ran |
| NUDT5 | GALNT3 | |

## Accelerating Drug Discovery

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