

FREQUENTLY ASKED QUESTIONS

How does KINOMEScan™ work?

The KINOMEScan platform utilizes a simple high-throughput competitive binding assay technology. A test compound is combined with human kinases tagged with DNA and an active-site directed ligand which is immobilized on a solid support. During equilibration, if the test compound binds to the kinase and either directly or indirectly competes with the ligand, fewer kinase molecules are able to interact with the active site directed ligand. Conversely, if the test compound does not compete, kinase molecules are free to bind to the immobilized ligand. The results are then read out by quantifying the amount of tagged kinase bound to the solid support using highly sensitive quantitative PCR.

Does profiling with KINOMEScan generate the expected results for known kinase inhibitors?

Yes. We have assessed the correlation between binding measured with our competition assays and inhibition observed in enzyme activity assays. K_d determinations were performed for known interactions of a panel of kinase inhibitors with reported IC50's or K_i 's below 1 μ M and compared them to published results. A high correlation between binding constants measured and published IC50 values was observed.

- Data from a number of known kinase inhibitors can be found in the supplementary tables from the Fabian *et al.* (2005), and Karaman *et al.* (2008) *Nature Biotechnology* publications, which are available for download at www.kinomescan.com/publications.

What are the benefits of KINOMEScan?

- Affords researchers a broad understanding of compound selectivity with 442 robust kinase assays, including the largest collection of lipid, tyrosine, and disease relevant mutant kinase assays available on the market.
- Flexible screening options including pre-configured kinase panels, custom panels for special projects, and open subscription agreements ensure that KINOMEScan has a solution that will fit your screening requirements.
- Economical pricing with innovative high throughput screening technology.
- Single assay platform for all kinases permit direct data comparison of compounds between kinases, and over time.
- Outstanding assay performance and data consistency with average Z' value of all assays exceeding 0.74, and correlation coefficients exceeding 0.96.



What is the concentration of ATP in your assays?

The KINOMEScan competition binding assay platform does not use ATP. No ATP is added.

Can you provide examples of current partners that utilize KINOMEScan?

KINOMEScan provides screening services to AstraZeneca, BMS, Cephalon, GSK, and Roche, among other leading organizations. These companies use KINOMEScan results to help drive medicinal chemistry and make decisions about their drug development programs.

How are KINOMEScan assays validated?

We use PCR to quantify binding affinity in each of our assays. This extremely sensitive measure gives the KINOMEScan platform a wide dynamic range. Binding constants from sub-nanomolar to micromolar affinities are measured accurately and consistently. Multiple control compounds are used for each experiment performed to ensure assay reliability and integrity. Additionally, assay data is continually monitored to ensure longitudinal uniformity.

How do I order?

The process is simple and fast. Request a quote for profiling using our online quote request tool available at www.kinomescan.com. Within a few hours you will receive a customized profiling quote from KINOMEScan that describes project scope, pricing, and compound submission guidelines. Sign and fax us your purchase order and ship compounds to our Compound Management Department. You will receive your study report within ten days of compound receipt.

Can I select which kinases I would like tested?

Yes. Through our online quote request tool, choose the *scan*ELECT panel and select any subset of kinases from our full panel of 442 kinases.

How do I supply compounds, and how much do you need?

Compound may be provided as a stock solution (50 ul of 10mM stock in DMSO), or dry (1-2 mg) for primary screens.

What is the final concentration of test compound in the assays?

Final test concentration is generally 10 μ M or 1 μ M but can be adjusted based on specific client requirements.

How are data reported? How long is data turnaround?

Assays are reported on a per interaction basis as one kinase and one compound in duplicate. Primary screening data are reported as a percent of control (P.O.C.) where lower numbers indicate stronger hits. Binding constants (K_d determinations) are calculated with a standard dose-response curve using the Hill equation and reported as a K_d . Screening data are immediately emailed to the customer as a .csv file, and are accompanied by a separate study report containing protocol details, color-enhanced compound assay matrix, plus TREEspot™ kinase interaction maps in .pdf file format. Screening data is available within two weeks or 10 business days from compound receipt.

What is library profiling?

The break-through technology utilized by KINOMEScan has not only dramatically reduced the cost of profiling – it has afforded researchers the most comprehensive access to the kinome. Scientists use these advantages to study any number of compounds against kinases of interest. However, on a larger scale, library profiling has enabled researchers to ask even better research questions in order to derive more value from their kinase discovery programs. Instead of testing many compounds against a single target (traditional HTS), library profiling exposes many compounds (1,000s to 10,000s) to as many targets as possible (e.g. the scanMAX panel). KINOMEScan's ability to screen more than 3,000 compounds per month against our full panel keeps timelines short for even the largest projects. With over 400 kinase assays for potential targets, KINOMEScan provides each compound many chances to succeed; annotating a chemical library also records activity against present and future targets. Below are just a few of the many advantages of library profiling:

- Targets that a chemical library can access are revealed.
- Leads revealed have a known selectivity profile; promiscuity can be assessed and considered against the desired disease indication.
- Unprecedented SAR from structurally related kinases or kinase subfamilies.
- Scaffolds can be grouped for multi-target leads and multi-indication programs.
- Broad multi-scaffold profiling reveals novel IP.
- Annotation records activity against future targets not yet described in literature.
- Cross therapy area leads: an oncology deck of compounds, for example, may hold leads for inflammation, CNS, and CV.

Can allosteric inhibitors be detected using KINOMEScan technology?

Yes. KINOMEScan is capable of detecting different classes of allosteric inhibitors:

- BIRB-796-like compounds which are ATP-competitive but bind only partially in the ATP site. Although not a true allosteric, they are sometimes referred to as allosteric inhibitors. This class of compounds is detected on the KINOMEScan platform.
- CI-1040-like compounds bind distal to the active site but within the kinase domain and are non-competitive with ATP. However affect active site conformation in such a way that binding of (at least some) other active-site binding inhibitors are prevented. There are only a few compounds of this class described in literature, including CI-1040, AZD-6244/ARRY-886, and BMS-345541, and KINOMEScan can detect binding of these compounds to their known targets.
- ON012380-like compounds are competitive with binding of protein/peptide substrate but have no effect on the active site. There is currently only one example of such a compound described in literature (ABL inhibitor ON012380). KINOMEScan assays will not detect this class of compound. However, some compounds which are competitive with protein/peptide substrate but not ATP will still affect active site conformation and therefore fall into the CI-1040-like compound class (as described above). In this case, they would be detected on the KINOMEScan platform.
- Inhibitors that bind to regulatory domains that interfere with kinase regulation but do not affect the conformation of the kinase domain itself will not be detected on the KINOMEScan platform. This compound class includes GNF-2, an ABL inhibitor that binds to the myristate site in ABL and inhibits auto-inhibition, and the PH-domain-binding AKT inhibitors. However, it is possible to build custom assays to explicitly query the allosteric sites targeted by ON012380-like and GNF-2-like inhibitors.

For additional answers, please contact sales@kinomescan.com.