KINOME*scan*™ Profiling Service

Kd Report

Compound Name	Kds Determined
JA-1-58	6

Requester: Glenn Micalizio

Company: Dartmouth University

Study Date: 03/08/2018

Report Date: 3/12/2018

Quote ID: KDELS07604A

Order ID: DTM005-01-s-00001

Product: KdELECT

Number of Kds Determined: 6

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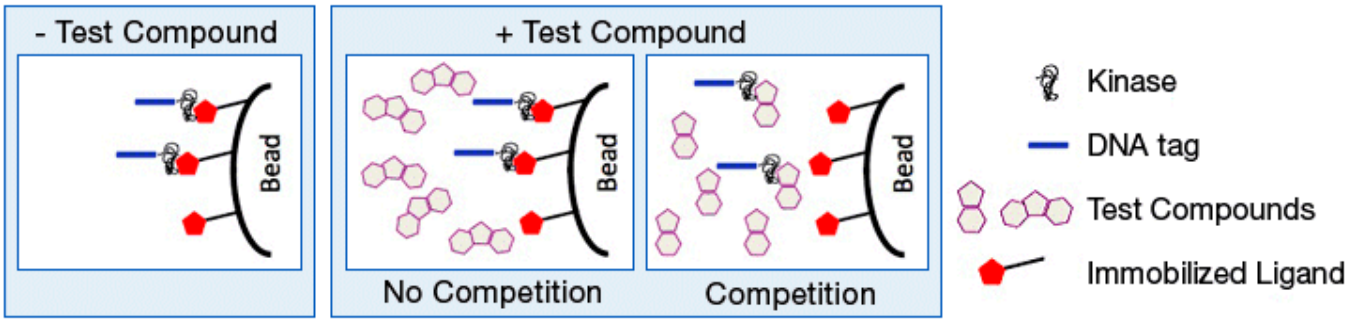
Technology Overview

KINOME*scan*™ is the industry's most comprehensive high-throughput system for screening compounds against large numbers of human kinases. Developed by DiscoverX, KINOME*scan*™ employs proprietary active-site dependent competition binding assays to determine how compounds bind to both intended and unintended kinases. In addition to helping keep discovery programs on track, KINOME*scan*™ can opportunistically identify unanticipated interactions that can expand the therapeutic utility of compounds or serve as advanced starting points for new programs.

How KINOME*scan*™ Works

KINOME*scan*™ is based on a competition binding assay that quantitatively measures the ability of a compound to compete with an immobilized, active-site directed ligand. The assay is performed by combining three components: DNA-tagged kinase; immobilized ligand; and a test compound. The ability of the test compound to compete with the immobilized ligand is measured via quantitative PCR of the DNA tag.



Protocol Description

Kinase assays. For most assays, kinase-tagged T7 phage strains were prepared in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage and incubated with shaking at 32°C until lysis. The lysates were centrifuged and filtered to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20% SeaBlock, 0.17x PBS, 0.05% Tween 20, 6 mM DTT). Test compounds were prepared as 111X stocks in 100% DMSO. Kds were determined using an 11-point 3-fold compound dilution series with three DMSO control points. All compounds for Kd measurements are distributed by acoustic transfer (non-contact dispensing) in 100% DMSO. The compounds were then diluted directly into the assays such that the final concentration of DMSO was 0.9%. All reactions performed in polypropylene 384-well plate. Each was a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 0.5 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

Compound Handling

An 11-point 3-fold serial dilution of each test compound was prepared in 100% DMSO at 100x final test concentration and subsequently diluted to 1x in the assay (final DMSO concentration = 1%). Most Kds were determined using a compound top concentration = 30,000 nM. If the initial Kd determined was < 0.5 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. A Kd value reported as 40,000 nM indicates that the Kd was determined to be >30,000 nM.

Binding Constants (Kds)

Binding constants (Kds) were calculated with a standard dose-response curve using the Hill equation:

$$\text{Response} = \text{Background} + \frac{\text{Signal} - \text{Background}}{1 + (\text{Kd}^{\text{Hill Slope}} / \text{Dose}^{\text{Hill Slope}})}$$

The Hill Slope was set to -1.

Curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm.

TREE*spot*™ Compound Profile Visualization Tool

TREE*spot*™ is a proprietary data visualization software tool developed by KINOME*scan*™. Visualize data online and create your own high resolution TREE*spot*™ interaction maps with our easy-to-use compound profile visualization tool. TREE*spot*™ is provided as a complimentary service to our clients. To access TREE*spot*™, please follow these directions:

Login: treespot.discoverx.com -- Username: treespot! -- Password: guest037
Instructions: treespot.discoverx.com/Help/TreeSpotHelpBasic.htm

References

KINOME*scan*™ and BROMO*scan*™ use the same assay technology. For a more detailed description of this assay technology, see:

- Fabian, M.A. *et al*. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* 23, 329-336 (2005).

To view kinase interactions for 38 well-known kinase inhibitors, see:

- Karaman, M.W. *et al*. A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* 26, 127-132 (2008).

For examples on how KINOME*scan* can opportunistically identify unanticipated therapeutically-beneficial interactions, see:

- Carter, T.A. *et al*. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc. Natl. Acad. Sci.* USA. 102, 11011-11016 (2005)

For more information on the Hill equation and the Levenberg-Marquardt algorithm, see:

- Hill, A. V. The possible effects of the aggregation of the molecules of hemoglobin on its dissociation curves. *J. Physiol. (Lond.).* 40, iv-vii (1910).
- Levenberg, K. A method for the solution of certain non-linear problems in least squares. *Q. Appl. Math.* 2, 164-168 (1944).

Select publications are available at www.discoverx.com.

DTM005-01-s-00001 Study Results

Table 1 - Matrix of Kds for DTM005-01-s-00001.

Target	JA-1-58
Gene Symbol	Kd (nM)
CLK1	1400
CLK2	2000
CLK4	350
DYRK1A	2000
JAK2(JH1domain-catalytic)	3700
LATS2	4600

Kd Legend

x<100nM	100nM≤x<1uM	x≥1uM	No Binding	Not Requested

DTM005-01-s-00001 Curve Images

Table 2 - Curve Images for DTM005-01-s-00001. The amount of kinase measured by qPCR (Signal; y-axis) is plotted against the corresponding compound concentration in nM in log10 scale (x-axis). Data points marked with an "x" were not used for Kd determination.