KINOME*scan*™ Profiling Service

Primary Screen Report

Compound Name	Screening Conc (nM)
JA-1-58	10000

Requester: Glenn Micalizio

Company: Dartmouth University

Study Date: 03/02/2018

Report Date: 03/04/2018

Quote ID: MAXXP11269A

Order ID: DTM003-01-p-00001

Product: scanMAX

Number of Targets Tested: 468

Compounds Screened: 1

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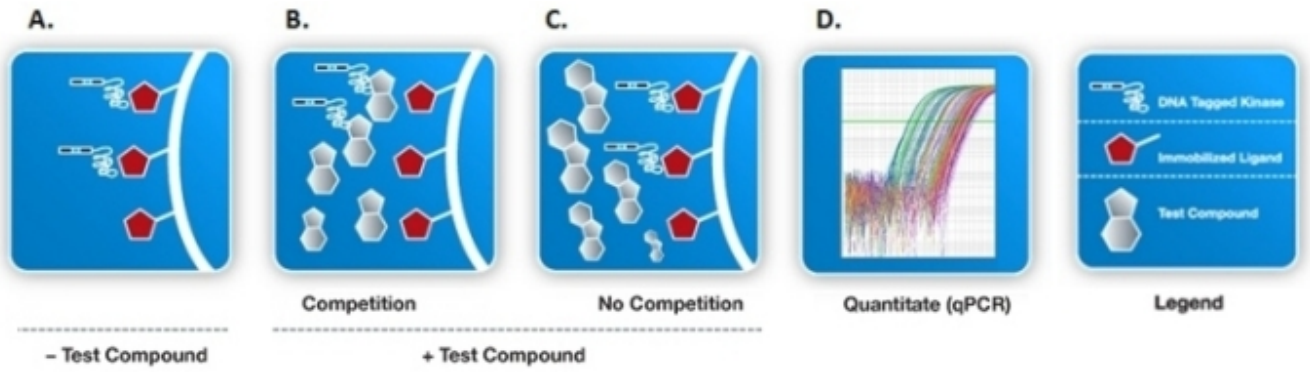
Technology Overview

The KINOME*scan*™ screening platform employs a novel and proprietary active site-directed competition binding assay to quantitatively measure interactions between test compounds and more than 450 human kinases and disease relevant mutant variants. This robust and reliable assay technology affords investigators the ability to extensively annotate compounds with accurate, precise and reproducible data. KINOME*scan*™ assays do not require ATP and thereby report true thermodynamic interaction affinities, as opposed to IC50 values, which can depend on the ATP concentration.

How KINOME*scan*™ Works

Compounds that bind the kinase active site and directly (sterically) or indirectly (allosterically) prevent kinase binding to the immobilized ligand, will reduce the amount of kinase captured on the solid support (A & B). Conversely, test molecules that do not bind the kinase have no effect on the amount of kinase captured on the solid support (C). Screening "hits" are identified by measuring the amount of kinase captured in test versus control samples by using a quantitative, precise and ultra-sensitive qPCR method that detects the associated DNA label (D). In a similar manner, dissociation constants (Kds) for test compound-kinase interactions are calculated by measuring the amount of kinase captured on the solid support as a function of the test compound concentration.



Protocol Description

Kinase assays. For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (6,000 x g) and filtered (0.2µm) 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 phage 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 40x stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in 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.

Percent Control (%Ctrl)

The compound(s) were screened at the concentration(s) requested, and results for primary screen binding interactions are reported as '% Ctrl', where lower numbers indicate stronger hits in the matrix on the following page(s).

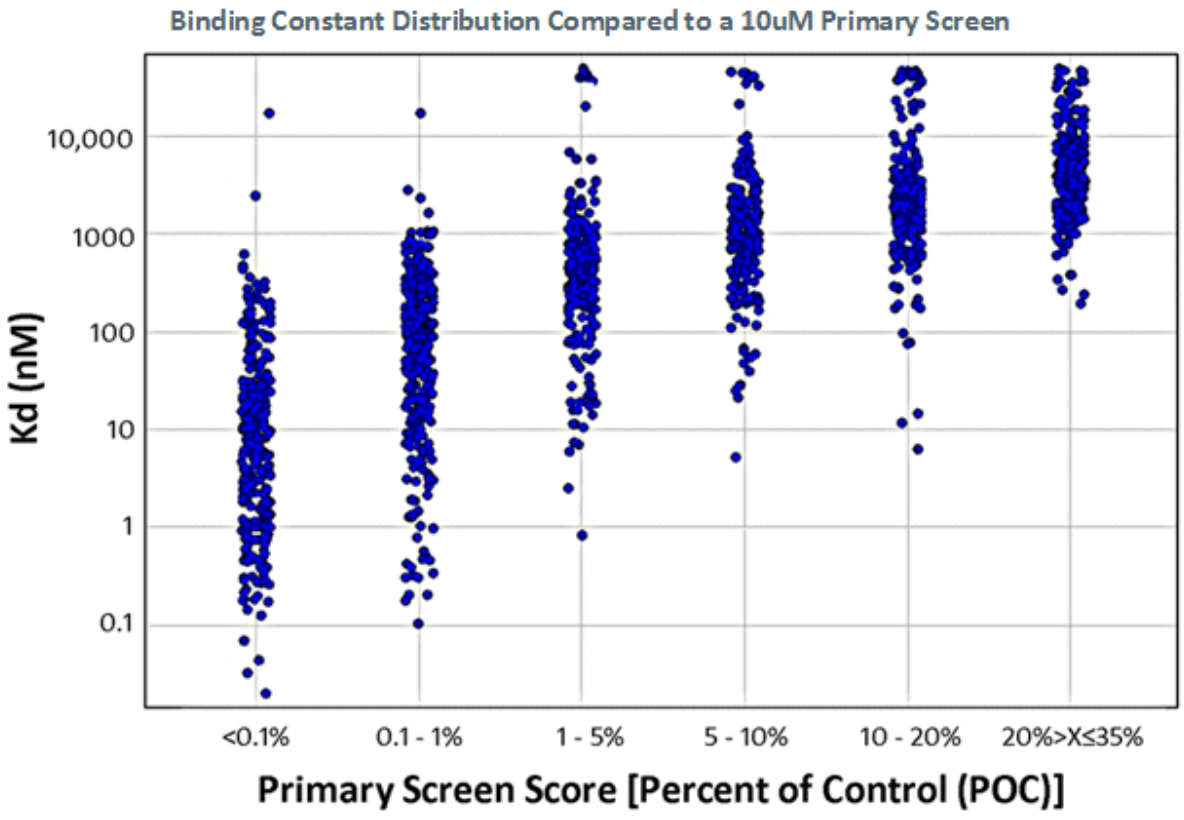
%Ctrl Calculation

$$\left(\frac{\text{test compound signal - positive control signal}}{\text{negative control signal - positive control signal}} \right) \times 100$$

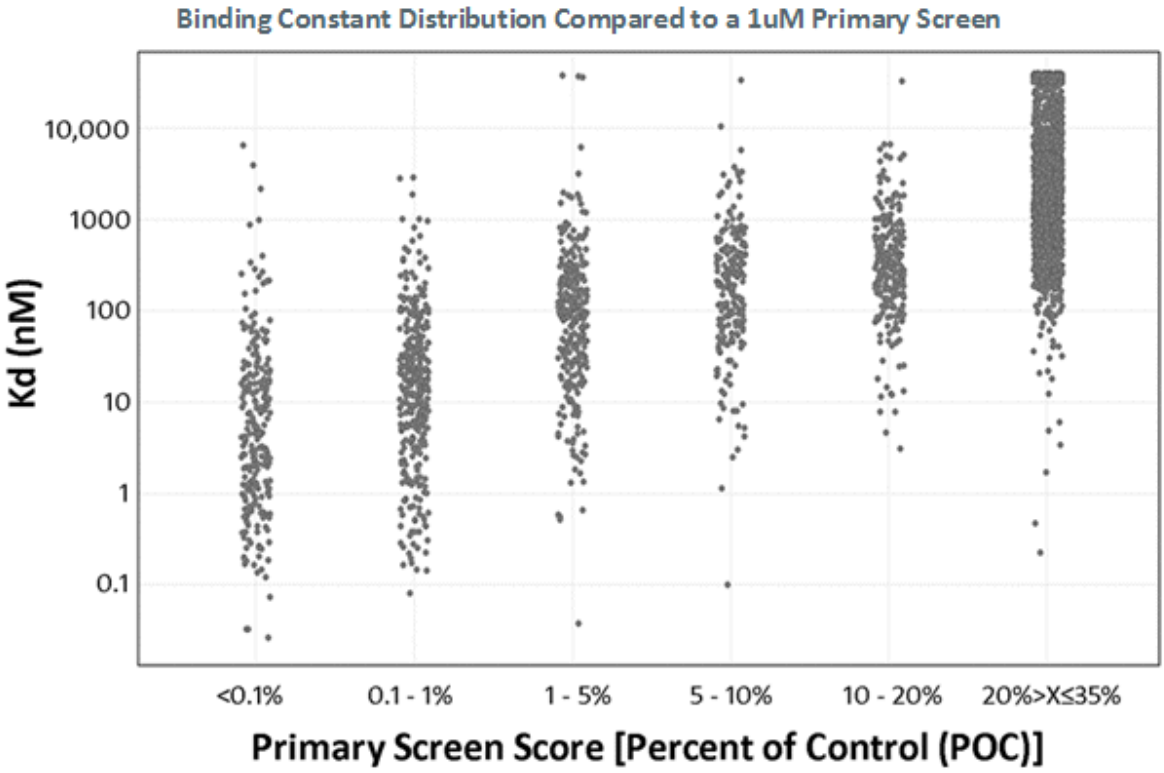
test compound = compound submitted by Dartmouth University
negative control = DMSO (100%Ctrl)
positive control = control compound (0%Ctrl)

Relationship between Binding Constant Distributions (Kds) &Single Concentration Primary Screen Values

Based on screening data from thousands of profiled compounds, a proportional relationship between primary screening results and corresponding compound/target affinities may be described. Evident in the correlation graph below is a range of binding constants (Kd values) for the indicated ranges of POC values with tighter binding (higher affinity) interactions associated with lower POC values and weaker binding (lower affinity) associated with higher POC values. This distribution of binding constants is characteristic of single concentration primary screens and underscores the importance of following up observed 'hits' or apparent high affinity interactions with quantitative binding constant determinations.



Data correlation between primary screening (10µM concentration) and binding constants (Kd values). Binding constants are correlated with primary screening results, where lower POC values are associated with low Kd values (higher affinity interactions).



Data correlation between primary screening (1µM concentration) and binding constants (Kd values). Binding constants are correlated with primary screening results, where lower POC values are associated with low Kd values (higher affinity interactions).

Selectivity Score (S-scores)

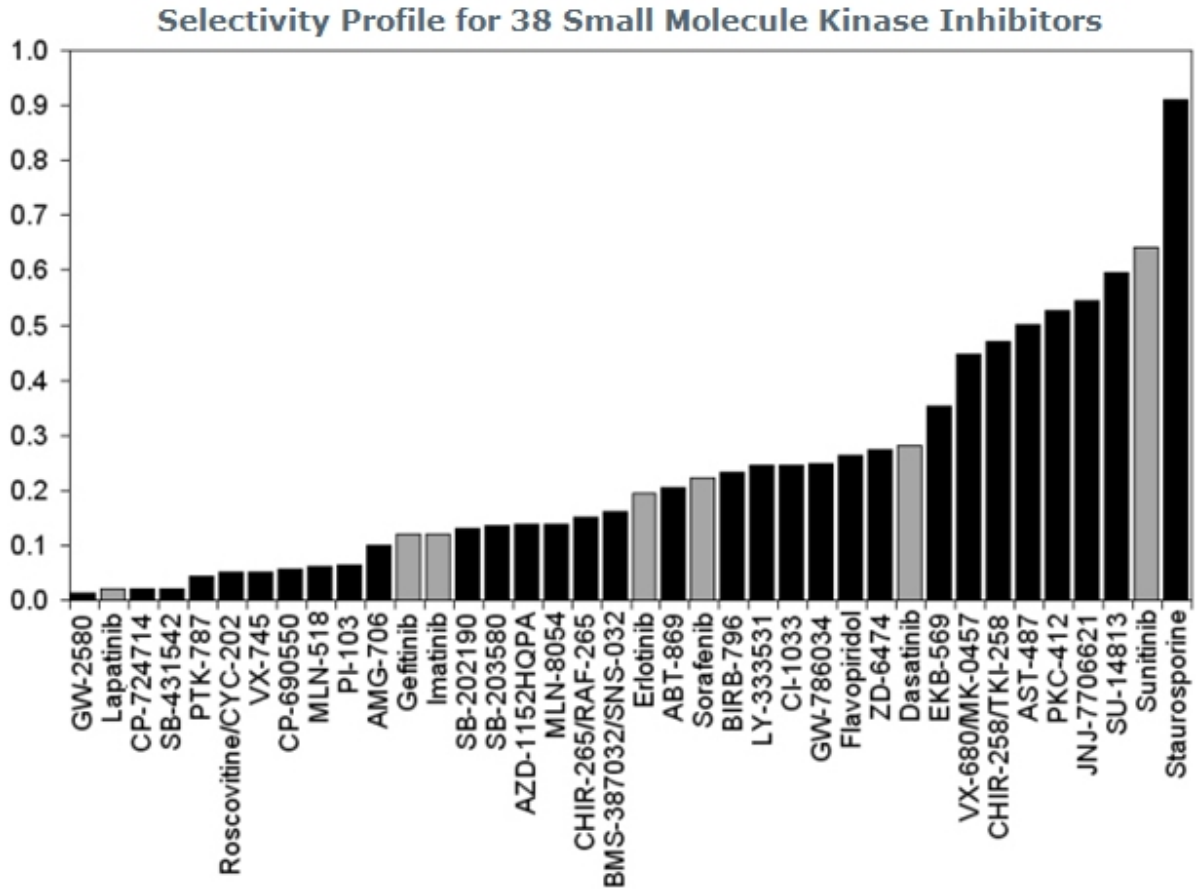
Selectivity Score or S-score is a quantitative measure of compound selectivity. It is calculated by dividing the number of kinases that compounds bind to by the total number of distinct kinases tested, excluding mutant variants.

S = Number of hits / Number of assays

This value can be calculated using %Ctrl as a potency threshold (below) and provides a quantitative method of describing compound selectivity to facilitate comparison of different compounds.

S(35) = (number of non-mutant kinases with %Ctrl <35)/(number of non-mutant kinases tested)
S(10) = (number of non-mutant kinases with %Ctrl <10)/(number of non-mutant kinases tested)
S(1) = (number of non-mutant kinases with %Ctrl <1)/(number of non-mutant kinases tested)

Using S-Score Data to Quantitate Selectivity



KINOMEscan's in vitro competition binding assay was used to evaluate 38 kinase inhibitors against a panel of 287 distinct human protein kinases (~55% of the predicted human protein kinome), and three lipid kinases. The compounds tested included 21 tyrosine kinase inhibitors, 15 serine-threonine kinase inhibitors, 1 lipid kinase inhibitor and staurosporine. S(35) = (number of non-mutant kinases with %Ctrl <35)/(290 kinases tested; 27 mutant variants were excluded from this analysis). Compounds approved for use in humans (as of August, 2007) are highlighted (gray bars).

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 interaction maps for 38 well-known kinase inhibitors and a more detailed discussion of selectivity scores, see:

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

Select publications are available at www.discoverx.com.

DTM003-01-p-00001 Study Results

Table 1 - Matrix of Compound Screen for DTM003-01-p-00001

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
AAK1	37
ABL1(E255K)-phosphorylated	67
ABL1(F317I)-nonphosphorylated	77
ABL1(F317I)-phosphorylated	73
ABL1(F317L)-nonphosphorylated	85
ABL1(F317L)-phosphorylated	86
ABL1(H396P)-nonphosphorylated	63
ABL1(H396P)-phosphorylated	73
ABL1(M351T)-phosphorylated	87
ABL1(Q252H)-nonphosphorylated	49
ABL1(Q252H)-phosphorylated	100
ABL1(T315I)-nonphosphorylated	86
ABL1(T315I)-phosphorylated	72
ABL1(Y253F)-phosphorylated	88
ABL1-nonphosphorylated	88
ABL1-phosphorylated	85
ABL2	98
ACVR1	89
ACVR1B	83
ACVR2A	95
ACVR2B	100
ACVRL1	95
ADCK3	83
ADCK4	100
AKT1	87
AKT2	100
AKT3	94
ALK	95
ALK(C1156Y)	72
ALK(L1196M)	81
AMPK-alpha1	100
AMPK-alpha2	83
ANKK1	55
ARK5	92
ASK1	100
ASK2	93
AURKA	100
AURKB	76
AURKC	87
AXL	82
BIKE	36
BLK	100
BMPR1A	86
BMPR1B	68
BMPR2	78
BMX	100
BRAF	92
BRAF(V600E)	90

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
BRK	87
BRSK1	88
BRSK2	93
BTK	100
BUB1	93
CAMK1	90
CAMK1B	74
CAMK1D	96
CAMK1G	95
CAMK2A	63
CAMK2B	72
CAMK2D	87
CAMK2G	98
CAMK4	66
CAMKK1	99
CAMKK2	96
CASK	55
CDC2L1	100
CDC2L2	97
CDC2L5	73
CDK11	60
CDK2	82
CDK3	100
CDK4	96
CDK4-cyclinD1	84
CDK4-cyclinD3	82
CDK5	93
CDK7	65
CDK8	87
CDK9	93
CDKL1	91
CDKL2	97
CDKL3	74
CDKL5	86
CHEK1	95
CHEK2	89
CIT	86
CLK1	8.2
CLK2	4.7
CLK3	73
CLK4	3.9
CSF1R	100
CSF1R-autoinhibited	61
CSK	96
CSNK1A1	82
CSNK1A1L	100
CSNK1D	81
CSNK1E	96
CSNK1G1	100

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
CSNK1G2	80
CSNK1G3	96
CSNK2A1	44
CSNK2A2	51
CTK	61
DAPK1	81
DAPK2	73
DAPK3	67
DCAMKL1	79
DCAMKL2	95
DCAMKL3	68
DDR1	93
DDR2	69
DLK	81
DMPK	76
DMPK2	89
DRAK1	76
DRAK2	71
DYRK1A	11
DYRK1B	30
DYRK2	67
EGFR	82
EGFR(E746-A750del)	89
EGFR(G719C)	90
EGFR(G719S)	93
EGFR(L747-E749del, A750P)	84
EGFR(L747-S752del, P753S)	75
EGFR(L747-T751del,Sins)	80
EGFR(L858R)	86
EGFR(L858R,T790M)	83
EGFR(L861Q)	76
EGFR(S752-I759del)	89
EGFR(T790M)	90
EIF2AK1	64
EPHA1	83
EPHA2	90
EPHA3	89
EPHA4	90
EPHA5	94
EPHA6	83
EPHA7	96
EPHA8	96
EPHB1	98
EPHB2	78
EPHB3	87
EPHB4	89
EPHB6	76
ERBB2	85
ERBB3	92

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
ERBB4	100
ERK1	85
ERK2	93
ERK3	78
ERK4	98
ERK5	99
ERK8	76
ERN1	73
FAK	87
FER	87
FES	85
FGFR1	86
FGFR2	94
FGFR3	90
FGFR3(G697C)	81
FGFR4	100
FGR	80
FLT1	95
FLT3	94
FLT3(D835H)	66
FLT3(D835V)	0
FLT3(D835Y)	45
FLT3(ITD)	86
FLT3(ITD,D835V)	49
FLT3(ITD,F691L)	72
FLT3(K663Q)	83
FLT3(N841I)	82
FLT3(R834Q)	71
FLT3-autoinhibited	74
FLT4	90
FRK	94
FYN	80
GAK	59
GCN2(Kin.Dom.2,S808G)	96
GRK1	66
GRK2	99
GRK3	36
GRK4	69
GRK7	79
GSK3A	83
GSK3B	78
HASPIN	34
HCK	100
HIPK1	33
HIPK2	46
HIPK3	51
HIPK4	68
HPK1	86
HUNK	100

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
ICK	71
IGF1R	83
IKK-alpha	64
IKK-beta	65
IKK-epsilon	71
INSR	91
INSRR	100
IRAK1	45
IRAK3	49
IRAK4	53
ITK	100
JAK1(JH1domain-catalytic)	100
JAK1(JH2domain-pseudokinase)	22
JAK2(JH1domain-catalytic)	12
JAK3(JH1domain-catalytic)	28
JNK1	53
JNK2	94
JNK3	91
KIT	96
KIT(A829P)	79
KIT(D816H)	12
KIT(D816V)	85
KIT(L576P)	94
KIT(V559D)	94
KIT(V559D,T670I)	92
KIT(V559D,V654A)	100
KIT-autoinhibited	69
LATS1	76
LATS2	6.6
LCK	92
LIMK1	91
LIMK2	91
LKB1	100
LOK	99
LRRK2	69
LRRK2(G2019S)	79
LTK	76
LYN	78
LZK	52
MAK	58
MAP3K1	64
MAP3K15	86
MAP3K2	76
MAP3K3	60
MAP3K4	77
MAP4K2	87
MAP4K3	100
MAP4K4	92
MAP4K5	95

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
MAPKAPK2	100
MAPKAPK5	52
MARK1	88
MARK2	82
MARK3	100
MARK4	91
MAST1	83
MEK1	66
MEK2	62
MEK3	47
MEK4	93
MEK5	44
MEK6	73
MELK	91
MERTK	94
MET	86
MET(M1250T)	83
MET(Y1235D)	94
MINK	75
MKK7	94
MKNK1	100
MKNK2	62
MLCK	100
MLK1	82
MLK2	64
MLK3	90
MRCKA	100
MRCKB	100
MST1	100
MST1R	77
MST2	67
MST3	92
MST4	77
MTOR	78
MUSK	100
MYLK	59
MYLK2	98
MYLK4	83
MYO3A	91
MYO3B	100
NDR1	81
NDR2	95
NEK1	84
NEK10	56
NEK11	100
NEK2	93
NEK3	81
NEK4	100
NEK5	78

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
NEK6	94
NEK7	81
NEK9	92
NIK	74
NIM1	100
NLK	98
OSR1	52
p38-alpha	91
p38-beta	84
p38-delta	71
p38-gamma	89
PAK1	95
PAK2	91
PAK3	93
PAK4	100
PAK6	98
PAK7	94
PCTK1	72
PCTK2	90
PCTK3	82
PDGFRA	72
PDGFRB	93
PDPK1	90
PFCDPK1(P.falciparum)	61
PFPK5(P.falciparum)	66
PFTAIRE2	90
PFTK1	92
PHKG1	84
PHKG2	69
PIK3C2B	83
PIK3C2G	55
PIK3CA	87
PIK3CA(C420R)	77
PIK3CA(E542K)	80
PIK3CA(E545A)	70
PIK3CA(E545K)	83
PIK3CA(H1047L)	82
PIK3CA(H1047Y)	89
PIK3CA(I800L)	49
PIK3CA(M1043I)	97
PIK3CA(Q546K)	66
PIK3CB	44
PIK3CD	92
PIK3CG	73
PIK4CB	39
PIKFYVE	50
PIM1	59
PIM2	14
PIM3	71

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
PIP5K1A	64
PIP5K1C	55
PIP5K2B	100
PIP5K2C	57
PKAC-alpha	79
PKAC-beta	100
PKMYT1	94
PKN1	81
PKN2	85
PKNB(M.tuberculosis)	79
PLK1	100
PLK2	89
PLK3	69
PLK4	65
PRKCD	74
PRKCE	46
PRKCH	92
PRKCI	62
PRKCQ	74
PRKD1	97
PRKD2	100
PRKD3	79
PRKG1	71
PRKG2	67
PRKR	87
PRKX	81
PRP4	63
PYK2	100
QSK	84
RAF1	96
RET	86
RET(M918T)	99
RET(V804L)	94
RET(V804M)	82
RIOK1	61
RIOK2	63
RIOK3	81
RIPK1	84
RIPK2	94
RIPK4	61
RIPK5	67
ROCK1	33
ROCK2	40
ROS1	84
RPS6KA4(Kin.Dom.1-N-terminal)	90
RPS6KA4(Kin.Dom.2-C-terminal)	72
RPS6KA5(Kin.Dom.1-N-terminal)	84
RPS6KA5(Kin.Dom.2-C-terminal)	85
RSK1(Kin.Dom.1-N-terminal)	100

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
RSK1(Kin.Dom.2-C-terminal)	58
RSK2(Kin.Dom.1-N-terminal)	46
RSK2(Kin.Dom.2-C-terminal)	76
RSK3(Kin.Dom.1-N-terminal)	84
RSK3(Kin.Dom.2-C-terminal)	83
RSK4(Kin.Dom.1-N-terminal)	73
RSK4(Kin.Dom.2-C-terminal)	78
S6K1	60
SBK1	62
SGK	67
SgK110	100
SGK2	55
SGK3	57
SIK	97
SIK2	93
SLK	97
SNARK	76
SNRK	73
SRC	84
SRMS	90
SRPK1	69
SRPK2	68
SRPK3	70
STK16	91
STK33	90
STK35	99
STK36	71
STK39	77
SYK	89
TAK1	69
TAOK1	72
TAOK2	99
TAOK3	83
TBK1	76
TEC	99
TESK1	87
TGFBR1	85
TGFBR2	86
TIE1	100
TIE2	84
TLK1	74
TLK2	90
TNIK	98
TNK1	67
TNK2	90
TNNI3K	82
TRKA	71
TRKB	54
TRKC	79

Table 1 - Assay Matrix (continued).

Target	JA-1-58
Gene Symbol	%Ctrl @ 10000nM
TRPM6	100
TSSK1B	97
TSSK3	87
TTK	80
TXK	93
TYK2(JH1domain-catalytic)	44
TYK2(JH2domain-pseudokinase)	67
TYRO3	87
ULK1	63
ULK2	69
ULK3	85
VEGFR2	83
VPS34	63
VRK2	59
WEE1	100
WEE2	100
WNK1	84
WNK2	70
WNK3	100
WNK4	81
YANK1	73
YANK2	100
YANK3	98
YES	92
YSK1	83
YSK4	41
ZAK	91
ZAP70	100

%Ctrl Legend

0≤x<.1	.1≤x<1	1≤x<10	10≤x<35	x≥35

S-score Results

Table 2 - S-score Table for DTM003-01-p-00001

Compound Name	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
JA-1-58	S(35)	13	403	10000	0.032
JA-1-58	S(10)	4	403	10000	0.01
JA-1-58	S(1)	0	403	10000	0

TREE*spot*™ Interaction Maps - Now Includes Mutant, Lipid, Atypical & Pathogen Kinase Dendrograms

As part of our ongoing effort to provide customers with the best possible data analysis tools, KINOME*scan*™ has developed an enhanced rendering of the human kinase dendrogram and allows, for the first time ever, to fully visualize compound interactions across our industry leading kinase panel, including clinically and biochemically relevant mutants, lipid, atypical, and pathogen kinases, plus a growing panel of activation-state specific assays.

TREE*spot*™ is an artistic representation of the human kinome phylogenetic tree based on extensive published research. We welcome your comments and feedback on this new visualization image. Please contact us at info@discoverx.com to tell us what you think.

Key Changes

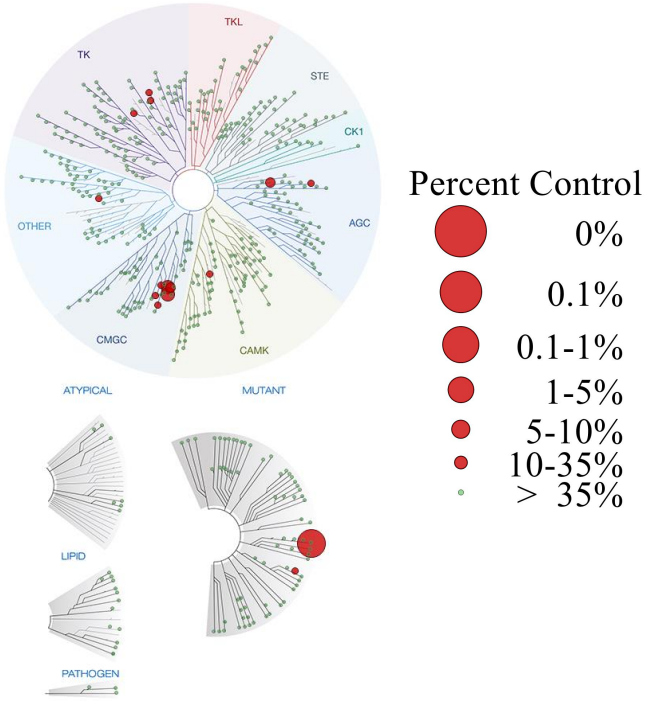
- More uniform format and presentation
- Kinase groups more clearly delineated
- Updated nomenclature for kinases

TREE*spot*™ is a proprietary data visualization software tool developed by KINOME*scan*. *Mutant and lipid kinases are not represented.* Kinases found to bind are marked with red circles, where larger circles indicate higher-affinity binding. Visualize data online and create your own high resolution TREE*spot*™ interaction maps with our easy-to-use compound profile visualization tool. Instructions and login credentials provided below.

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

Table 3 - TREE*spot*™ Interaction Maps for DTM003-01-p-00001

JA-1-58 @ 10000nM



Available Follow-up Screening Services

LeadHunter™ Discovery Services offers a suite of investigative tools that enable detailed biochemical characterization of the interaction between inhibitors and their targets. The thermodynamic, kinetic, and structural information provided by these tools enables a detailed comparison of inhibitors from common or distinct lead series and facilitates the interpretation of data from downstream cellular and *in vivo* pharmacology models. These services are now available for both kinases and for bromodomain-containing proteins.

K_dELECT Obtain quantitative binding affinities for compound-kinase interactions

***Kd*ELECT™** - a powerful follow up service to quantify binding affinity of compound-kinase interactions identified in primary (single concentration) screens. Inhibitor binding constants (Kd values) are calculated from duplicate 11-point dose-response curves under optimized conditions that generate true thermodynamic Kd values which facilitate direct comparison of inhibitor affinity across kinases. Learn more >>

PathHunter® cell-based compound screening & profiling services

PathHunter inCell assays and screening services are a powerful follow up solution to KINOME*scan*™ *in vitro* biochemical studies for obtaining the maximum level of information about inhibitor function, potency and selectivity in a more physiological context.