

## Primary Screen Report

**Requester:** John Doe

**Company:** Company X

**Study Date:** 6/15/2011

**Report Date:** 6/15/2011

**Quote ID:** ZZZ001-01-p

**Order ID:** ZZZ001-01-p-00001

**Product:** scanMAX

**Number of Kinases Tested:** 317

**Compounds Screened:** 3

Compound Name	Screening Conc (nM)
Gleevec	10000
GW-2016	10000
SU-11248	10000



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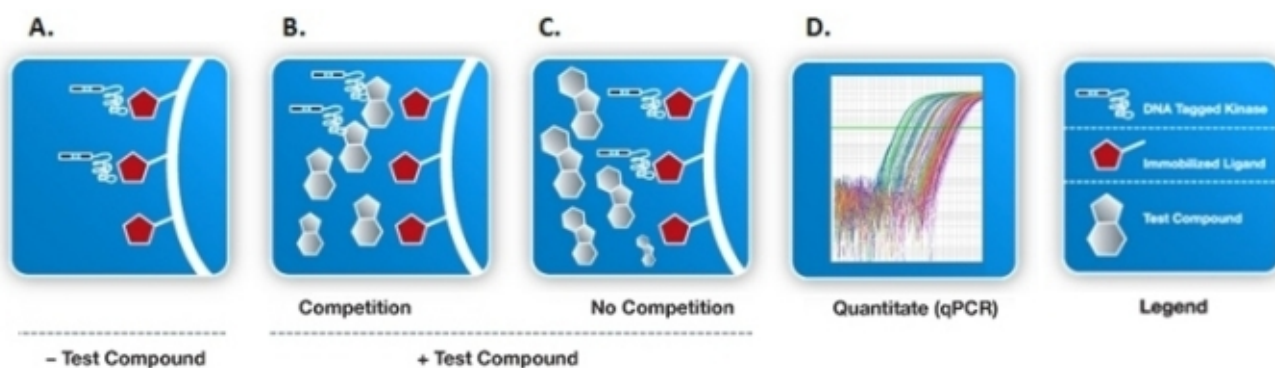
San Diego, CA 92121

## Technology Overview

The KINOMEScan™ 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. KINOMEScan™ 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 KINOMEScan™ 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 0.04 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

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

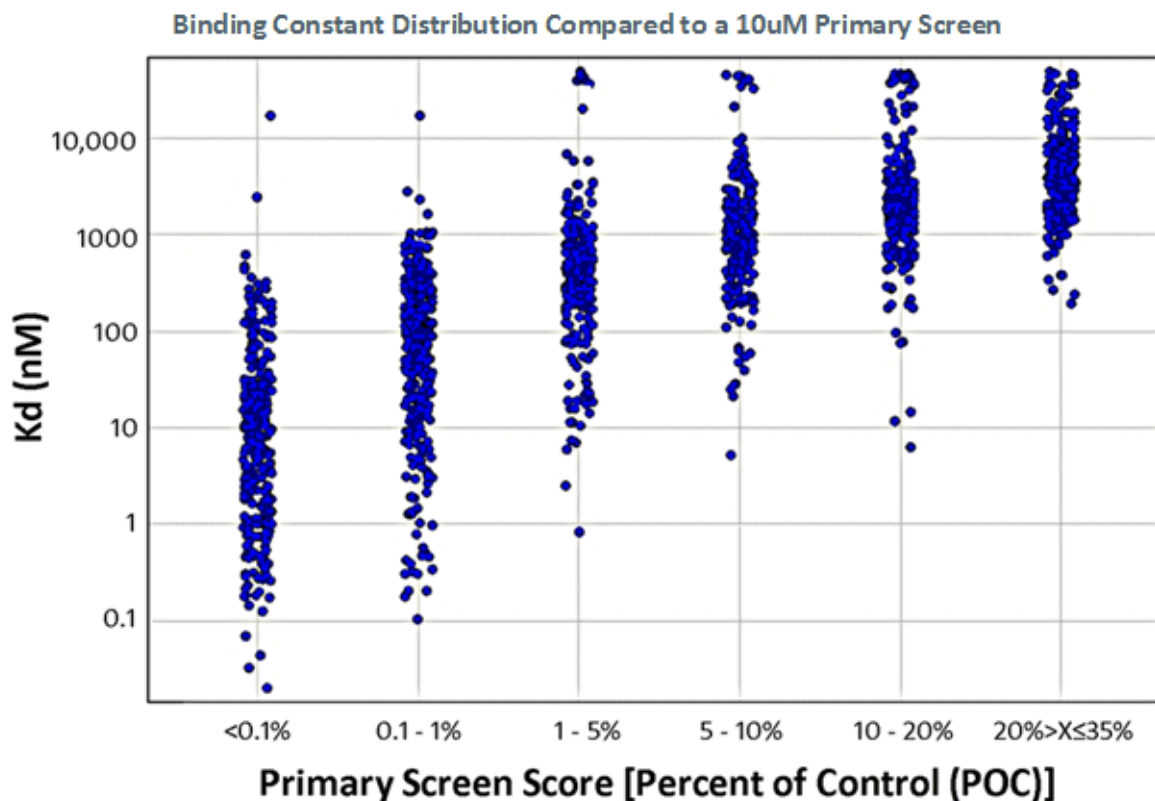
*test compound* = compound submitted by KINOMEScan

*negative control* = DMSO (100%Ctrl)

*positive control* = control compound (0%Ctrl)

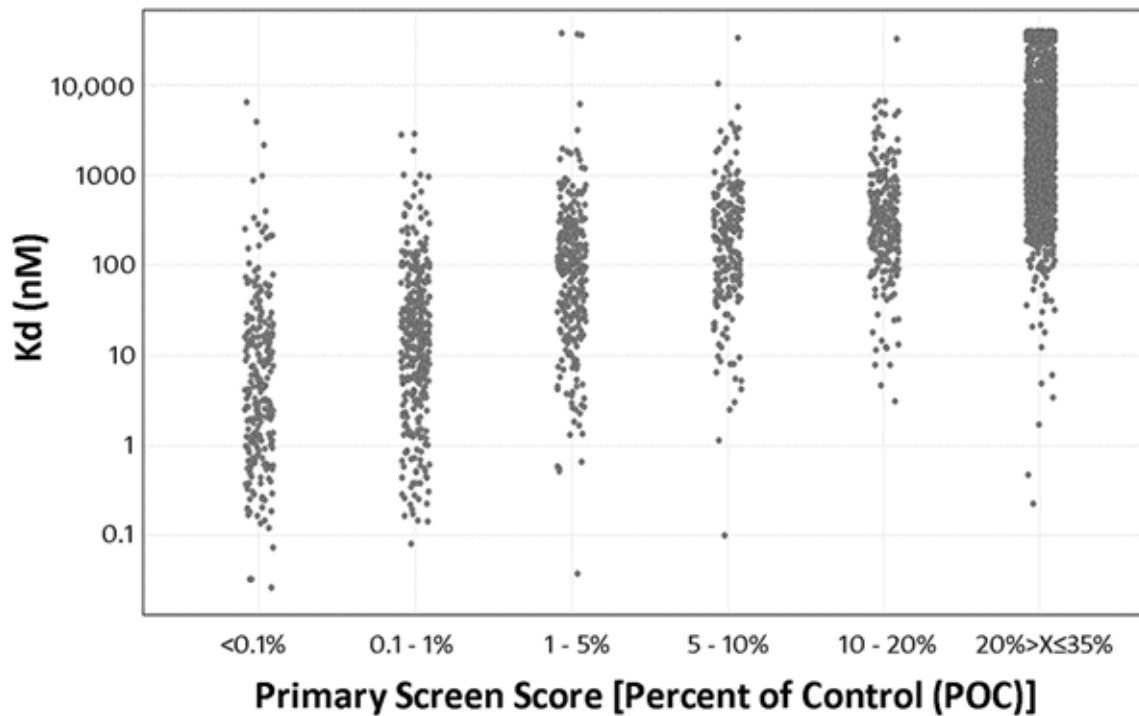
### Relationship between Binding Constant Distributions (Kds) & Single Concentration Primary Screen Values

Based on screening data from thousands of profiled compounds, a proportional relationship between primary screening results and corresponding compound/kinase 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).*

**Binding Constant Distribution Compared to a 1uM Primary Screen**



*Data correlation between primary screening (1 $\mu$ M concentration) and binding constants ( $K_d$  values). Binding constants are correlated with primary screening results, where lower POC values are associated with low  $K_d$  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.

$$S = \text{Number of hits} / \text{Number of assays}$$

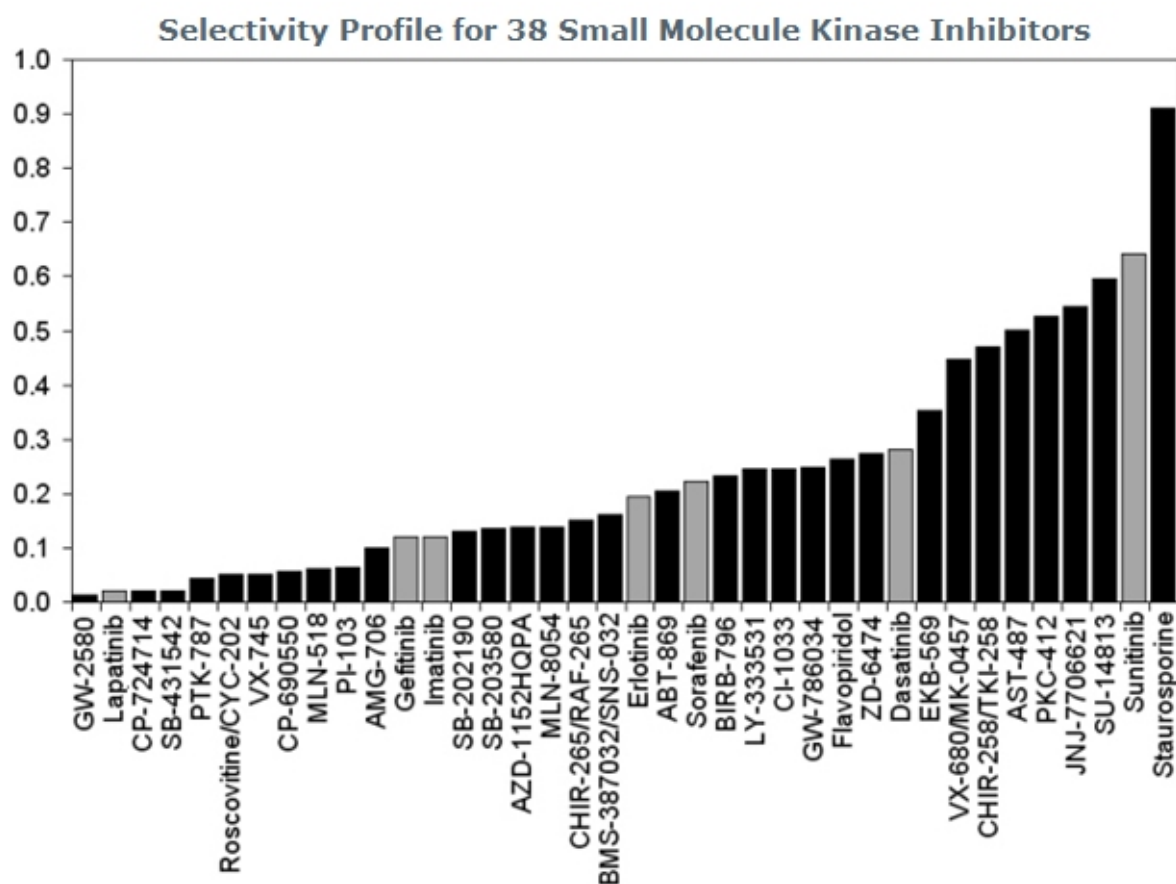
This value can be calculated using %Ctrl as a potency threshold (below) and provides a quantitative method of describing compound selectivity to facilitate comparison of different compounds.

$$S(35) = (\text{number of non-mutant kinases with \%Ctrl} < 35) / (\text{number of non-mutant kinases tested})$$

$$S(10) = (\text{number of non-mutant kinases with \%Ctrl} < 10) / (\text{number of non-mutant kinases tested})$$

$$S(1) = (\text{number of non-mutant kinases with \%Ctrl} < 1) / (\text{number of non-mutant kinases tested})$$

## 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.  $S(35) = (\text{number of non-mutant kinases with \%Ctrl} < 35) / (290 \text{ kinases tested}; 27 \text{ mutant variants were excluded from this analysis})$ . Compounds approved for use in humans (as of August, 2007) are highlighted (gray bars).

## References

For a more detailed description of KINOMEScan's assay technology, see:

- Fabian *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.kinomescan.com](http://www.kinomescan.com).

## ZZZ001-01-p-00001 Study Results

Table 1 - Matrix of Compound Screen for ZZZ001-01-p-00001

Kinase Target	Gleevec	GW-2016	SU-11248
KINOMEScan Gene Symbol	%Ctrl @ 10000nM	%Ctrl @ 10000nM	%Ctrl @ 10000nM
AAK1	34	75	0.85
ABL1	1.4	62	8.6
ABL1(E255K)	3.4	87	25
ABL1(H396P)	1.5	80	8
ABL1(M351T)	1.4	75	6.5
ABL1(Q252H)	1	84	12
ABL1(T315I)	34	79	0.1
ABL1(Y253F)	2.2	80	9.4
ABL2	0.4	77	20
ACVR1	100	91	48
ACVR1B	88	87	71
ACVR2A	85	73	88
ACVR2B	88	80	90
ACVRL1	90	89	100
ADCK3	87	99	100
ADCK4	96	87	100
AKT1	87	90	100
AKT2	100	100	30
AKT3	100	100	100
ALK	79	82	0
AMPK-alpha1	93	95	1.1
AMPK-alpha2	98	84	0.75
ANKK1	55	97	0
ARK5	84	87	0.1
ASK1	91	75	100
AURKA	47	75	27
AURKB	93	100	0.5
AURKC	62	76	0.45
AXL	89	80	0
BIKE	82	100	0.15
BLK	0.8	49	0.6
BMPR1A	100	98	99
BMPR2	88	84	2.2
BMX	84	96	91
BRAF	19	100	100
BRAF(V600E)	20	72	94
BRK	84	94	48
BRSK1	100	100	11
BRSK2	100	87	14
BTK	100	88	61
CAMK1	71	95	8.4
CAMK1D	17	97	4.6
CAMK1G	67	83	2.1
CAMK2A	25	83	3
CAMK2B	50	91	12
CAMK2D	15	100	6.7
CAMK2G	11	96	5.1
CAMK4	100	91	0.35

Table 1 - Assay Matrix (continued).

Kinase Target	Gleevec	GW-2016	SU-11248
KINOMEScan Gene Symbol	%Ctrl @ 10000nM	%Ctrl @ 10000nM	%Ctrl @ 10000nM
CAMKK1	1.4	89	6.4
CAMKK2	2.1	100	12
CDC2L1	100	87	100
CDC2L2	100	81	100
CDK11	34	69	97
CDK2	100	80	92
CDK3	100	85	83
CDK5	100	100	39
CDK7	100	92	0.9
CDK8	73	65	61
CDK9	100	92	86
CHEK1	100	93	0.1
CIT	70	57	6.2
CLK1	29	71	0.55
CLK2	30	98	4.9
CLK3	68	78	43
CLK4	12	91	0.65
CSF1R	0	87	0
CSK	69	94	57
CSNK1A1L	100	100	0.85
CSNK1D	38	88	0.95
CSNK1E	19	60	0.05
CSNK1G1	95	80	1.1
CSNK1G2	68	100	0.8
CSNK1G3	100	98	0.35
CSNK2A1	74	100	0.55
CSNK2A2	73	100	3
DAPK1	87	91	0.1
DAPK2	94	84	0.5
DAPK3	59	81	0.2
DCAMKL1	100	89	2
DCAMKL2	92	88	15
DCAMKL3	100	100	0.05
DDR1	0.2	63	3.1
DDR2	7.4	100	45
DLK	100	100	0.35
DMPK	100	84	100
DMPK2	100	96	50
DRAK1	5.2	77	0
DRAK2	78	88	0.1
DYRK1B	100	95	6
EGFR	88	0	69
EGFR(E746-A750del)	83	0.9	66
EGFR(G719C)	60	0	26
EGFR(G719S)	77	0.05	16
EGFR(L747-E749del, A750P)	28	0.05	48
EGFR(L747-S752del, P753S)	57	0.2	7.4
EGFR(L747-T751del,Sins)	42	0.1	9.3
EGFR(L858R)	67	0	12

Table 1 - Assay Matrix (continued).

Kinase Target	Gleevec	GW-2016	SU-11248
KINOMEScan Gene Symbol	%Ctrl @ 10000nM	%Ctrl @ 10000nM	%Ctrl @ 10000nM
EGFR(L861Q)	54	0	9.6
EGFR(S752-I759del)	81	0.3	54
EPHA1	86	86	47
EPHA2	52	86	80
EPHA3	39	90	32
EPHA4	86	100	65
EPHA5	89	88	51
EPHA6	80	82	27
EPHA7	100	100	23
EPHA8	7.2	98	78
EPHB1	91	94	8.9
EPHB2	68	17	45
EPHB3	82	94	74
EPHB4	93	96	41
ERBB2	91	14	85
ERBB4	42	0.35	60
ERK1	100	100	100
ERK2	100	100	99
ERK3	100	95	56
ERK4	100	89	100
ERK5	93	93	41
ERK8	100	99	66
FAK	59	100	1.2
FER	91	89	26
FES	78	94	27
FGFR1	80	84	5.4
FGFR2	100	100	4.4
FGFR3	97	90	3
FGFR3(G697C)	82	83	3.1
FGFR4	99	75	41
FGR	20	92	3.6
FLT1	78	100	0
FLT3	76	74	0.05
FLT3(D835H)	68	88	0
FLT3(D835Y)	37	82	0
FLT3(ITD)	44	96	0
FLT3(N841I)	81	69	0
FLT4	85	100	0
FRK	14	83	13
FYN	23	86	11
GAK	5.1	84	0
GCN2(Kin.Dom.2,S808G)	94	94	0.4
GSK3A	86	96	81
GSK3B	100	100	82
HCK	22	68	6.6
HPK1	77	78	0.55
IGF1R	100	90	10
IKK-epsilon	100	98	11
INSR	92	82	0.95

Table 1 - Assay Matrix (continued).

Kinase Target	Gleevec	GW-2016	SU-11248
KINOMEScan Gene Symbol	%Ctrl @ 1000nM	%Ctrl @ 1000nM	%Ctrl @ 1000nM
INSRR	100	100	0.1
IRAK3	42	65	11
ITK	96	89	0.1
JAK1(JH2domain-pseudokinase)	95	100	0
JAK2(JH1domain-catalytic)	94	81	8.2
JAK3(JH1domain-catalytic)	100	94	8.1
JNK1	14	81	67
JNK2	75	97	78
JNK3	5.6	79	39
KIT	0	75	0
KIT(D816V)	6.2	77	0.2
KIT(V559D)	0	64	0
KIT(V559D,T670I)	20	96	0
KIT(V559D,V654A)	0.3	90	0
LATS1	100	94	1.2
LATS2	98	78	1.8
LCK	1	63	1.8
LIMK1	88	72	93
LIMK2	85	67	96
LKB1	76	100	8.9
LOK	79	7.4	2.7
LTK	88	61	29
LYN	3.2	78	5.6
MAP3K4	98	88	42
MAP4K3	100	100	0.7
MAP4K4	87	89	2.8
MAP4K5	92	80	0.05
MAPKAPK2	100	100	100
MAPKAPK5	95	100	100
MARK1	100	95	10
MARK2	8.8	78	6.5
MARK3	100	100	0.65
MARK4	91	99	8.9
MEK1	100	82	0.3
MEK2	97	79	0
MEK3	90	82	82
MEK4	77	70	40
MEK6	100	93	44
MELK	29	81	0.65
MERTK	91	100	0
MET	94	97	39
MKNK1	79	82	37
MKNK2	57	100	42
MLCK	100	100	0
MLK1	96	100	29
MLK2	100	74	64
MLK3	95	100	22
MRCKA	100	67	84
MRCKB	100	69	84

Table 1 - Assay Matrix (continued).

Kinase Target	Gleevec	GW-2016	SU-11248
KINOMEScan Gene Symbol	%Ctrl @ 10000nM	%Ctrl @ 10000nM	%Ctrl @ 10000nM
MST1	99	100	3
MST2	4.8	100	0
MST3	100	100	0
MST4	100	100	0
MUSK	97	91	0.6
MYLK	95	95	0.15
MYLK2	85	100	0.3
MYLK4	53	73	0
MYO3A	94	100	36
MYO3B	100	100	24
NDR2	100	100	4.4
NEK1	93	99	85
NEK2	96	63	0.55
NEK5	100	100	81
NEK6	51	100	46
NEK7	41	90	22
NEK9	82	90	87
NLK	100	70	92
p38-alpha	100	100	100
p38-beta	77	98	100
p38-gamma	49	60	100
PAK1	88	96	68
PAK2	100	96	63
PAK3	100	94	1.6
PAK4	72	91	20
PAK6	59	93	18
PAK7	70	100	17
PCK1	92	95	0
PCK2	96	94	16
PCK3	96	93	21
PDGFRA	0.15	93	0
PDGFRB	0	74	0
PDPK1	57	87	33
PFTK1	90	91	11
PHKG1	84	91	1.2
PHKG2	39	94	6.8
PIK3CA	89	94	40
PIK3CA(E545K)	95	94	49
PIM1	71	82	50
PIM2	100	100	100
PIM3	89	100	12
PIP5K1A	66	74	0.2
PIP5K2B	100	88	0
PKAC-alpha	100	99	7.4
PKAC-beta	100	100	100
PKMYT1	75	100	100
PKN1	100	69	4.2
PKN2	100	98	24
PLK1	100	100	72

Table 1 - Assay Matrix (continued).

Kinase Target	Gleevec	GW-2016	SU-11248
KINOMEScan Gene Symbol	%Ctrl @ 10000nM	%Ctrl @ 10000nM	%Ctrl @ 10000nM
PLK3	42	91	98
PLK4	14	88	0
PRKCD	100	85	39
PRKCE	93	89	76
PRKCH	100	88	100
PRKCC	100	96	24
PRKD1	99	94	0.55
PRKD2	100	90	0.15
PRKD3	98	99	0.65
PRKG1	93	98	88
PRKG2	100	100	85
PRKR	68	85	1.6
PRKX	98	94	66
PYK2	93	100	0.5
RAF1	11	56	100
RET	85	90	0
RET(M918T)	92	80	0
RIOK1	94	86	0.2
RIOK3	89	84	0.1
RIPK1	86	100	0.35
RIPK2	80	25	59
ROS1	56	96	100
RPS6KA4(Kin.Dom.1-N-terminal)	99	68	0.4
RPS6KA4(Kin.Dom.2-C-terminal)	91	87	13
RPS6KA5(Kin.Dom.1-N-terminal)	99	86	8.1
RPS6KA5(Kin.Dom.2-C-terminal)	75	76	6.5
RSK1(Kin.Dom.1-N-terminal)	68	79	0
RSK1(Kin.Dom.2-C-terminal)	82	100	24
RSK2(Kin.Dom.1-N-terminal)	84	88	1.3
RSK3(Kin.Dom.1-N-terminal)	71	75	0.3
RSK3(Kin.Dom.2-C-terminal)	98	100	35
RSK4(Kin.Dom.1-N-terminal)	100	100	5.2
RSK4(Kin.Dom.2-C-terminal)	98	98	46
SIK	85	91	34
SIK2	75	67	8.6
SLK	98	40	0.15
SNARK	93	79	0.3
SRC	73	97	14
SRMS	78	45	24
SRPK1	56	90	0
SRPK2	98	100	1.6
STK16	61	61	9.2
STK33	85	98	0.4
STK36	92	95	88
SYK	74	100	99
TEC	100	88	82
TESK1	93	67	78
TGFBR1	77	100	89
TGFBR2	100	98	76

Table 1 - Assay Matrix (continued).

Kinase Target	Gleevec	GW-2016	SU-11248
KINOMEScan Gene Symbol	%Ctrl @ 10000nM	%Ctrl @ 10000nM	%Ctrl @ 10000nM
TIE1	97	76	38
TIE2	91	76	72
TLK1	100	100	0.8
TLK2	100	100	0.2
TNIK	69	100	6.3
TNK1	29	92	7.8
TNK2	84	96	20
TNNI3K	27	71	100
TRKA	86	91	4.2
TRKB	99	100	2
TRKC	75	76	8.8
TSSK1B	100	89	18
TTK	82	91	3.6
TXK	70	56	56
TYK2(JH1 domain-catalytic)	87	93	11
TYRO3	100	87	7
VEGFR2	57	100	0
WEE1	100	100	9.3
YANK2	86	100	100
YANK3	81	74	100
YES	41	80	0.7
YSK1	82	100	0.4
ZAK	21	78	49
ZAP70	100	97	100

%Ctrl Legend

0≤x<1	1≤x<10	10≤x<35	x≥35
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## S-score Results

Table 2 - S-score Table for ZZZ001-01-p-00001

Compound Name	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
Gleevec	S(35)	41	290	10000	0.141
Gleevec	S(10)	19	290	10000	0.066
Gleevec	S(1)	7	290	10000	0.024
GW-2016	S(35)	6	290	10000	0.021
GW-2016	S(10)	3	290	10000	0.01
GW-2016	S(1)	2	290	10000	0.007
SU-11248	S(35)	182	290	10000	0.628
SU-11248	S(10)	140	290	10000	0.483
SU-11248	S(1)	82	290	10000	0.283



## TREEspot™ 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, KINOMEScan™ 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.

TREEspot™ 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 [sales@kinomescan.com](mailto:sales@kinomescan.com) to tell us what you think.

### Key Changes

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

TREEspot™ is a proprietary data visualization software tool developed by KINOMEScan. *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 TREEspot™ interaction maps with our easy-to-use compound profile visualization tool. [Instructions and login credentials provided below.](#)

**Login:** [treespot.kinomescan.com](http://treespot.kinomescan.com) -- **Username:** kinomescan! -- **Password:** guest037

**Instructions:** [treespot.kinomescan.com/Help/TreeSpotHelpBasic.htm](http://treespot.kinomescan.com/Help/TreeSpotHelpBasic.htm)

Table 3 - TREEspot™ Interaction Maps for ZZZ001-01-p-00001

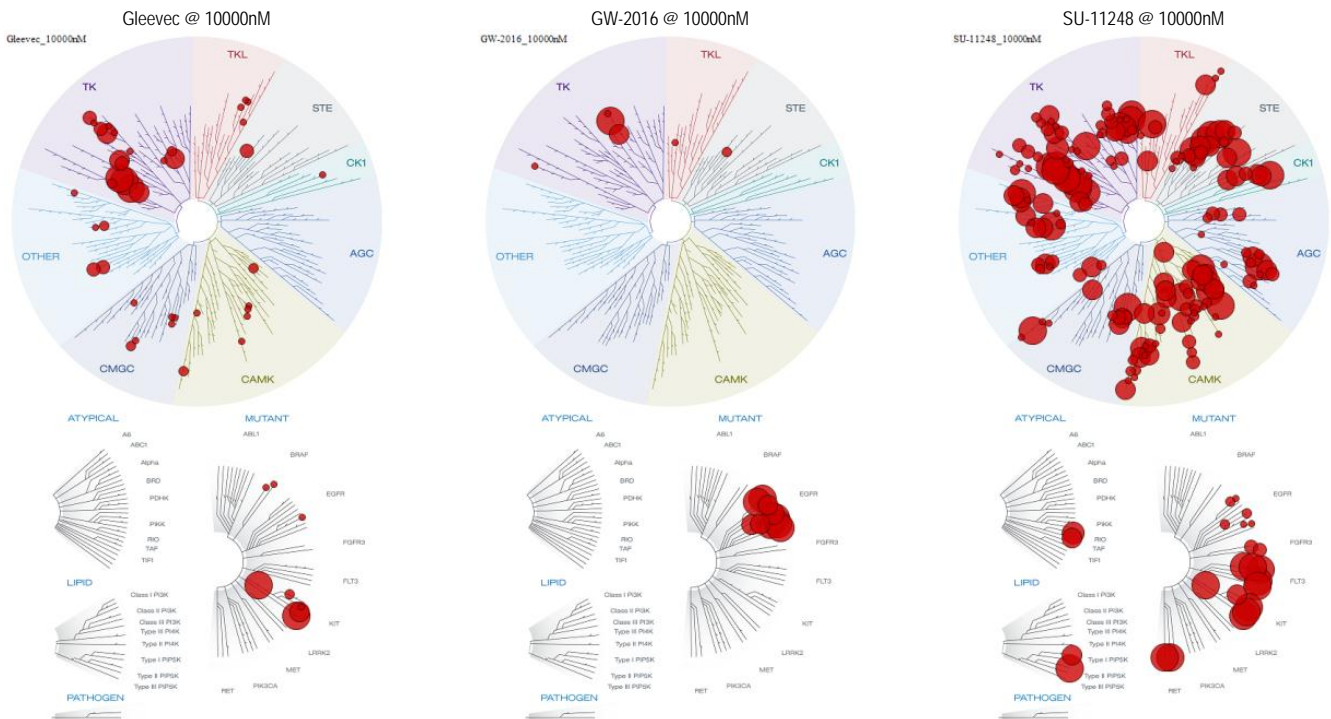
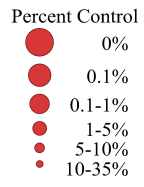


Table 3 - TREEspot™ Interaction Maps (continued).



## Available Follow-up Screening Services

KINOMEScan™ 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.

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### Obtain quantitative binding affinities for compound-kinase interactions

#### ***K<sub>d</sub>*ELECT**

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

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### Explore inhibitor classification & binding mode

#### **scanMODE**

**scanMODE™** - a novel biochemical tool consisting of activation state-specific assay pairs that may be used to functionally characterize inhibitor binding mode and annotate compound profile data by measuring phosphorylation state-dependence of inhibitor affinity. **scanMODE™** is performed as a panel of K<sub>d</sub> determinations against phosphorylated/non-phosphorylated ABL assay pairs and reported together. [Learn more >>](#)

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### **NEW** Reversibility and Dissociation Kinetics Studies

Irreversible, covalent inhibitors and reversible inhibitors that dissociate slowly from a kinase target can have unusual properties in both cellular and *in vivo* pharmacology models, which, in the absence of target dissociation data can make interpretation of pharmacology results difficult, particularly when multiple inhibitors are being compared. KINOMEScan™ now offers a dissociation kinetics service that classifies inhibitors as irreversible, reversible-slow dissociation, or reversible-rapid dissociation. [Learn more >>](#)

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### **Now Available!** PathHunter® cell-based compound screening & profiling services

PathHunter cell-based kinase assays and screening services are a powerful follow up solution to KINOMEScan™ *in vitro* biochemical studies for obtaining the maximum level of information about inhibitor function, potency and selectivity in a more physiological context. [View available assays for profiling >>](#)