

## ounds in HeLa live cell, with or without washout

Kinase	Labeling Site	No washout	
		Alectinib	SRPKIN-1
		5μM	5μM
CLK3	Lys2	ND	ND
EphB2	Activation Loop	ND	ND
STLK6	Lys1	ND	ND
YSK1	Lys1	ND	ND
ZC3/MINK	Lys1	ND	ND
CHK2	Lys2	>99	44.7
CaMKK2	Lys1	>98	68.8
CHK2	Lys1	>98	31.5
SRPK1, SRPK2	Lys1	>95	>95
CaMKK2	Lys2	96.1	27.6
FER	Lys1	95.4	11.0
SRPK1	Lys2	92.4	>99
CaMK4	Lys2	91.9	89.6
ACK	Lys1	90.4	27.3
CaMK2d	Lys1	85.1	-16.6
RSK1 domain1	Lys2	84.7	-4.9
RSK2 domain1	Activation Loop	84.3	-10.8
RSK2 domain1	Lys2	84.2	-8.9
RSK1 domain1	Activation Loop	83.6	3.7
RSK1 domain1	Lys1	83.5	5.6
RSK1 domain1, RSK2 domain1, RSK3 domain1	Lys2	82.9	-4.3
CaMK2g	Lys1	81.9	-32.1
FER	Activation Loop	80.9	6.6
RSK1 domain2	Lys2	78.5	13.4
MELK	Lys2	78.5	7.2
STLK5	Activation Loop	76.6	43.4
IRAK1	Lys2	76.5	16.4
CaMKK1	Lys2	74.3	2.7
EphB4	Activation Loop	73.7	-15.8
KHS2	Lys1	70.7	-5.6
CaMK2a, CaMK2b, CaMK2d, CaMK2g	Lys2	68.0	-15.5
RSK2 domain2	Lys2	66.9	9.2
STLK3	Lys2	66.1	47.7
RSK2 domain1	ATP Loop	65.9	-19.5
LOK	Lys2	65.8	40.5
SLK	Lys2	61.4	37.1
FAK	Activation Loop	57.0	24.5
FAK	Lys1	54.8	11.5
IRAK4	Lys2	53.2	24.0
SNRK	Lys1	52.5	-1.8
SNRK	Lys2	51.6	18.4
PHKg2	Lys1	51.4	16.3
IRAK4	Lys1	51.0	10.3
MAP3K2, MAP3K3	Lys2	46.9	25.5

SGK3	Lys1	46.5	-1.7
MAP3K4	Lys2	46.5	25.9
PIP4K2C	ATP	44.3	29.9
OSR1	Lys2	43.9	26.7
KHS1	Lys1	42.2	-5.2
PIP4K2C	ATP	42.2	33.5
ILK	Protein Kinase Domain	41.9	32.9
ULK3	Lys2	41.1	8.7
p70S6Kb	Lys2	40.6	19.1
KSR1	Activation Loop	39.3	18.3
LOK	Lys1	38.5	-14.9
BRAF	Lys2	37.8	10.9
PIK3C2B	ATP	37.7	24.0
GCK	Lys2	37.3	23.5
ULK3	Lys1	34.1	10.1
RSKL1	ATP	33.9	32.7
NEK3	Activation Loop	33.6	5.0
ARAF	Lys2	33.4	-4.7
STLK6	Lys2	33.1	-17.1
LATS1	Lys1	32.6	8.5
SGK3	Lys2	31.7	16.6
PAN3	ATP	30.9	29.7
TAK1	Lys2	30.4	15.9
LYN	Activation Loop	30.2	-18.1
MAP2K7	Lys2	29.9	18.5
PLK1	Lys2	28.6	19.9
LATS2	Lys1	28.5	28.1
IKKe, TBK1	Lys2	27.5	14.3
MAP2K5	Lys2	27.4	26.9
TBK1	Lys1	26.7	4.1
NEK8	Lys2	26.6	5.3
PFTAIRE1	Lys2	26.3	21.4
ZC1/HGK, ZC2/TNIK, ZC3/MINK	Lys2	26.2	-3.6
KSR1, KSR2	Activation Loop	25.6	14.6
Erk5	Lys2	25.6	-0.3
AMPKa1, AMPKa2	Lys2	25.0	12.4
PFTAIRE1	Lys1	24.5	3.5
LATS1	Lys2	24.4	5.0
Wnk1, Wnk2, Wnk3	Lys2	24.4	8.2
ULK1	Lys2	24.2	12.8
MET	Lys1	23.6	-1.2
MAPKAPK3	ATP Loop	22.7	-4.8
LYN	Lys1	22.7	6.4
PKD3	Lys1	21.7	26.8
Erk2	Lys2	20.2	15.1
GCK	Lys1	19.6	-11.0
p38a	Protein Kinase Domain	18.6	4.4
PCTAIRE1, PCTAIRE3	Lys2	18.5	3.3
CHK1	Lys2	18.4	7.7

p70S6K	Lys2	17.7	24.5
Erk1	Lys2	17.7	14.0
MLK3	Lys2	17.7	5.8
p38a	Lys2	17.4	17.0
MSK2 domain1	Lys2	17.2	-3.7
p38d, p38g	Lys2	16.9	26.5
FGFR1, FMS, PDGFRb, TYRO3	Lys1	16.6	-12.5
PCTAIRE2, PCTAIRE3	Lys1	15.5	-10.0
MAP2K3	Lys1	15.4	14.1
NEK6, NEK7	Lys2	15.1	2.0
AKT2, AKT3	ATP Loop	13.8	-6.5
MAP3K5	Lys2	13.5	-2.6
JNK1, JNK2, JNK3	Lys2	13.4	6.2
MAP2K5	Lys1	12.8	-7.4
CaMK1d	Lys1	12.3	-2.8
HER2/ErbB2	Lys1	11.6	-1.6
AMPKa1, AMPKa2	Lys1	11.4	-11.1
CSK	Activation Loop	10.7	2.7
PLK1	Lys1	10.4	-1.5
ZAK	Lys1	9.4	-2.7
ILK	Lys1	8.6	0.2
PCTAIRE1	Lys1	7.7	2.1
MST1, MST2	Lys2	7.2	0.4
AMPKa1	ATP Loop	7.0	-17.2
AKT1	ATP Loop	6.8	-13.3
MLK4	Lys2	6.5	4.3
CaMK1a	Lys1	6.2	-12.2
EGFR	Activation Loop	5.2	-7.7
Wnk1, Wnk2, Wnk4	Activation Loop	5.1	-2.0
PI4KB	ATP	4.8	-26.0
CaMK1d	Lys2	4.2	1.9
Wnk1, Wnk2	ATP Loop	4.0	-2.7
PI4KB	ATP	3.9	-5.2
SMG1	ATP	3.7	-0.3
MAP2K1, MAP2K2	Lys1	3.4	-2.5
PKN1	Lys2	3.0	19.4
NEK9	Lys2	2.7	-2.6
MAP2K3	Lys2	2.6	-11.4
MAP2K6	Lys1	2.2	14.8
CDK5	Lys2	1.8	-2.3
CDK2	Lys1	1.3	6.8
CCRK	Lys2	1.1	-5.3
MSK1 domain1	Lys2	0.8	7.9
MAP3K2	Lys1	0.4	1.5
CDC2	Lys2	-0.5	-1.9
IKKa	Lys2	-1.6	5.5
p38b	Protein Kinase Domain	-1.9	8.8
NEK9	Lys1	-2.0	-3.9
CDC2	Lys2	-2.7	-5.4







CDK5	Lys1	-3.4	-16.2
LATS2	Lys2	-4.0	-5.1
MAP3K3	Lys1	-4.1	-34.5
MAP2K1, MAP2K2	Lys2	-4.2	-3.7
NEK1	Lys2	-4.4	2.4
ITPK1	ATP	-4.6	-16.0
NEK4	Lys2	-5.8	1.3
PEK	Lys2	-6.0	28.0
NDR1	Lys1	-7.1	22.0
NDR1	Lys2	-7.7	-4.7
MAP2K4	Lys2	-9.1	-1.0
ABL, ARG	Lys1	-9.3	15.9
NEK7	Lys1	-9.3	-12.9
MAP2K1	Lys2	-9.4	11.8
PIP5K3	ATP	-9.6	-0.5
AurA	Lys1	-9.6	-9.1
SGK	Lys1	-10.2	-6.4
CDK7	Lys1	-11.8	-21.0
JAK1	Protein Kinase Domain	-12.0	-46.8
CK2a1	Protein Kinase Domain	-12.2	-1.1
PKD2	Lys1	-12.3	3.6
IKKe	Lys1	-12.7	-63.5
MAP2K4	Lys1	-12.7	20.4
MAP3K6	Lys2	-12.9	-2.0
SLK	Lys1	-13.4	-20.5
IKKb	Lys1	-13.7	23.5
MST1	Lys1	-13.8	-17.1
PIK3C3	ATP	-13.8	-21.3
AurA	Lys2	-14.5	-12.9
MET	Activation Loop	-15.1	19.7
CSK	Lys1	-15.7	-27.7
MAP3K1	Lys2	-16.6	-27.3
BARK1	Lys2	-17.1	-7.8
CDK2	Lys2	-17.5	-0.2
PKN1	Lys1	-17.8	16.0
ABL, ARG	Activation Loop	-18.2	3.5
NuaK2	Lys1	-19.9	-76.7
JAK1 domain2	Lys1	-20.6	-10.7
CHK1	Protein Kinase Domain	-21.2	2.7
NDR2	Lys2	-21.6	-18.6
MAP2K6	Lys2	-21.6	-0.9
MASTL	ATP Loop	-21.7	-3.6
MAP3K15, MAP3K5, MAP3K6	Lys1	-21.9	-15.3
SMG1	ATP	-22.0	-17.4
IKKb	Lys2	-22.2	16.2
NDR2	Lys1	-23.1	11.8
PKR	Lys2	-24.4	3.4
EphA2	Activation Loop	-24.6	-11.4
HER2/ErbB2	Activation Loop	-24.8	-12.8

NLK	Lys2	-24.8	7.7
CDK7	Lys2	-25.7	-5.9
MST4, YSK1	Lys2	-26.5	10.7
YANK3	Lys2	-29.4	-9.6
MARK3, MARK4	Lys1	-31.4	-8.8
PIK3C3	ATP	-33.5	-25.7
MARK1	Lys1	-34.1	-27.8
AurA, AurB, AurC	ATP Loop	-34.2	-18.6
MARK2	Lys1	-35.0	-3.0
MST2	Lys1	-36.1	-7.1
MASTL	Lys1	-38.1	-13.3
MARK3	Lys1	-39.4	-18.4
ATM	ATP	-39.7	-15.1
AGK	ATP	-40.0	-18.1
TLK1	Lys2	-40.3	-16.7
FYN, SRC, YES	Activation Loop	-40.7	-39.4
p70S6K, p70S6Kb	ATP Loop	-41.1	-13.7
CASK	Lys1	-41.8	-20.4
TLK2	Lys2	-42.0	-19.6
MAST3	Lys2	-42.6	-12.6
PKD1, PKD2	Lys2	-43.1	-2.1
CHED	Lys2	-43.2	-4.6
GCN2	Lys2	-44.7	-10.5
MARK1, MARK2	Lys1	-45.0	-6.9
ROCK1, ROCK2	Lys2	-45.5	29.2
PKR	Activation Loop	-46.0	-26.0
GPRK5	Lys2	-46.7	-39.1
MAST1, MAST2	Lys2	-46.7	3.5
TLK1	Lys1	-46.7	-28.6
MST3	Lys2	-47.1	-6.2
PIK3CB	ATP	-47.5	-8.3
NuaK1	Lys1	-50.7	-31.0
TLK2	Lys1	-52.2	-31.9
GCN2	Lys1	-52.6	-26.7
MST4	Lys1	-52.7	-37.0
CDK11, CDK8	Lys2	-52.7	20.7
MST3	Lys1	-52.8	-22.2
ANPa	Lys2	-53.7	-5.3
MARK2, MARK3	Lys2	-54.4	4.1
FRAP	ATP	-54.7	-37.8
PI4KA, PI4KAP2	ATP	-56.6	-29.0
eEF2K	ATP	-56.8	-16.9
ROCK1	Protein Kinase Domain	-56.9	0.0
MPSK1	Lys2	-60.2	-3.4
MLKL	Lys1	-64.5	-32.2
PKCi	Lys2	-69.7	-1.5
PKD3	Lys2	-72.1	-6.3
JAK1 domain2	Activation Loop	-72.8	-36.4
TAO1, TAO3	Lys2	-75.6	1.7

CDK10	Lys2	-77.8	-17.0
AurB	Lys1	-79.5	-48.2
CK1d, CK1e	Lys2	-80.1	-11.6
ATR	ATP	-82.1	6.1
CK1a	Lys2	-82.7	-0.9
GSK3A	Lys2	-85.7	-19.0
IRE1	Lys2	-89.0	-22.9
PKN2	Lys2	-90.1	3.3
VRK2	Lys2	-94.4	-19.8
PKCi	Lys1	-95.6	-27.0
CK1g1	Lys2	-95.6	-41.0
GSK3B	Lys2	-102.4	-21.5
CDK9	Lys2	-113.0	19.1
DNAPK	ATP	-125.3	-35.0
EGFR	Lys1	-133.8	-11.8
PRP4	Lys2	-135.8	-28.1
TAO2	Lys2	-139.7	-32.7
CRK7	Lys2	-140.8	9.4
CK1g2	Lys2	-143.8	-32.6
PIP4K2A	ATP	-154.2	-32.9
DNAPK	ATP	-169.9	-37.7
PRP4	Protein Kinase Domain	-191.7	-34.0
PRPK	ATP Loop	-200.7	-36.9
PITSLRE	Lys2	-230.7	-71.8
CLK3	ATP Loop	-335.4	-129.1

### Labeling Site Key

Lys1	Conserved Lysine 1
Lys2	Conserved Lysine 2
ATP Loop	ATP binding loop
Activation Loop	Activation loop
ATP	ATP site in non-canonical kinase (e.g. lipid kinase)
Protein Kinase Domain	Other lysine within kinase domain, <b>possibly not in ATP binding site</b>
Other	Labeling of residue outside of the protein kinase domain, <b>possibly not in ATP binding site</b>

	>90% Inhibition
	75 - 90% Inhibition
	50 - 75% Inhibition
	35 - 50% Inhibition
	No change
	>100% increase in MS signal (>2 fold increase)
ND	Not determined

Data points inhibited >35% & not considered significant are left uncolored

**Note:** This KiNativ dataset is the result of an analysis of duplicate treated samples and either duplicate or quadruplicate control samples. The % changes in MS signals being reported are statistical

significant (Student T-test score  $<0.04$ ). Additionally, the KiNativ method is performed using biological matrices (cells/tissues), which can be inherently variable, followed by a complex procedure of sample preparation and mass spec analysis. Thus, we recommend the use of independent, biological replicates to determine the inter-sample variability of the dataset (i.e., repeating the experimental conditions more than one time and with an independent sample of the biological matrix in order to validate the dataset). In addition, we strongly recommend that customers obtain supporting data through additional independent KiNativ studies or through orthogonal approaches prior to making critical decisions.

The data reported was performed in a non-GLP manner and was not intended for regulatory submission. It was generated to provide scientific data for informational purposes only.

KiNativ project managers are available to discuss the results and assist customers in understanding strengths and limitations of this KiNativ dataset. ActivX Biosciences, Inc. bears no responsibility for decisions made by customers based on this KiNativ dataset or for any experimental suggestions made by KiNativ project managers.

Washout

SRPKIN-1 5μM

-0.6

11.3

-7.9

-19.9

-22.4

30.1

20.1

20.7

>95

17.1

-0.8

>98

-10.1

16.0

-0.9

7.9

11.1

3.4

15.9

25.3

10.5

-7.4

8.7

-4.5

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-41.4

-7.1

3.9

-30.4

-3.8

1.1

-8.3

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ND

10.7  
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-29.5  
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-4.0  
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ND

-16.8  
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-0.4  
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