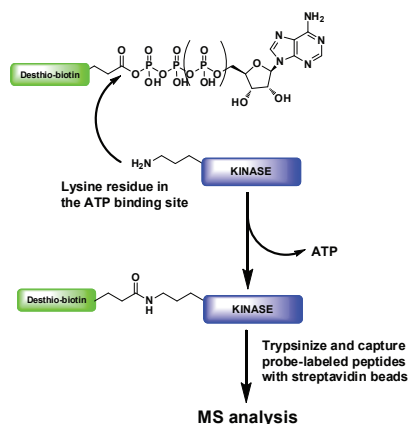


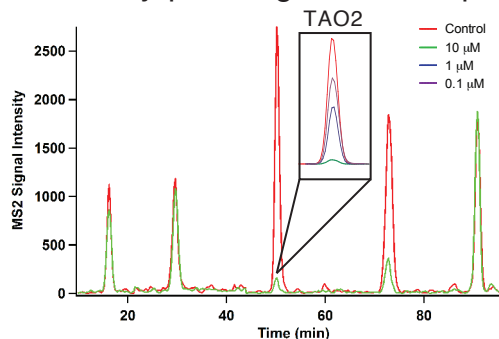
Lead Optimization

Native kinase profiling and inhibitor selectivity screen

KiNativ™ is a kinase screening platform that utilizes chemical probes to label, selectively purify and detect native kinases in samples ranging from cell lysates to animal tissues. Off-target profiling against full-length kinases, with endogenous post-translation modifications and protein:protein interactions still intact, ensures that selectivity data are truly representative of a compound's *in vivo* activity.



Selectivity profiling of staurosporine



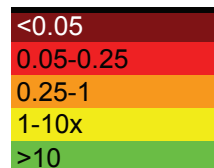
- Targeted LC-MS² permits evaluation of up to 180 native kinases per run (>200 kinases/proteome)
- Automated sample preparation and targeted data collection provide quantitative, reproducible data
- Better correlation with *in vivo* results compared to recombinant kinase assays

Selectivity profiling results using KiNativ™

Kinase name	ATP Kd	Staurosporine	Dasatinib	Tarceva	BIRB796	Sorafenib	Gleevec
ABL1/2	5.2	13	0.0067/0.0034	>10	>10	>10	0.21/0.15
ACK	22	0.062	0.033	>10	>10	>10	>10
AurA/B/C	9.5	0.076	>10	7.4	>10	>10	>10
AurB	31	0.03/0.012	>10/4	6.4/2.6	>10/4	>10/4	>10/4
BRAF	6.1	>10	3.2	>10	>10	>10	>10
ETK	86	0.91	0.016	>10	>10	>10	>10
CDK5	13	0.64	>10	>10	>10	>10	>10
CDK8/11	19	2.2	>10	>10	>10	>10	11
CSK	17	2.9/2.4	0.015/0.012	>10	>10	>10	>10
EGFR	18	>10	4.7/4.1	0.3/0.19	>10	>10	>10
EphA1	1.7	4.3	0.022	>10	>10	>10	>10
EphA2	22	11	0.017	>10	>10	>10	>10
EphB2	5.2	10	0.014	>10	>10	>10	>10
EphB4	8.5	>10	0.0028	5.2	>10/4	>10/4	>10
FGR	2.6	0.92/0.37	0.0068/0.0027	>10/4	>10/4	>10/4	>10/4
FYN	ND	0.27/ND	ND	5.8/ND	ND	>10/ND	ND
GCK	26	0.64	>10	>10	>10	>10	>10
GCN2	92	1.3/1.1	>10	0.76/0.6	>10	>10	>10
HCK	10	0.43	0.015	>10	>10	>10	>10
ILK	57	>10	0.24	1.1	>10	>10	>10
IRAK3	360	0.012	10	>10	>10	>10	ND
JAK1 (Domain 2)	1.8	0.21/0.051	5.3/1.3	>10/2.5	>10/2.5	>10/2.5	>10/2.5
JNK1/2/3	19	9.2	>10	>10	0.087	>10	8.6
JNK2	ND	3.1	>10	>10	0.029	>10	5.3
KHS1	9.6	0.12	0.4	>10	>10	>10	>10
KHS1/2	7.8	0.23	2.2	>10	>10	>10	>10
LOK	12	0.012	10	1.2	1	5.3	>10
LYN	1	0.41	0.0031	>10	>10	>10	>10
MAP2K1	8.1	0.49/0.2	10/4	>10/4	>10/4	ND	>10/4
MAP2K5	2.6	2.6/1.4	0.058/0.037	10	>10	>10	>10
MAP3K1	64	10	0.49	0.63	>10	0.82	>10
MAP3K2	7.9	1	6.5	>10	>10	>10	>10
MAP3K4	20	>10	0.51	>10	>10	>10	>10
MET	74	0.63/0.67	>10	3.5/2.4	>10	>10	>10
MINK,HGK,TNIK (ZC	7.6	0.11	>10	>10	1.1	>10	>10
MLKL	150	>10	3.2	>10	>10	>10	>10
p38a	570	>10	0.77	>10	0.19	2.4	>10
p38a (MAPKAPK2/3)	4.5	>10	2.4	>10	>10	>10	>10
p38dig	300	1.3	>10	>10	0.45	>10	>10
PIK3C3	97	>10	>10	>10	>10	>10	9.5
PIP5K2c	63	>10	>10	3.7	>10	>10	1.5
PIP5K3	7	10	>10	>10	>10	>10	4.2
PYK2	70	0.2	>10	>10	9	>10	>10
QSK	20	0.12	0.39	>10	>10	>10	>10
SLK	3.8	0.0091	>10	0.58	>10	>10	>10
SRC	9.8	0.89	0.0033	>10	>10	>10	>10
STLK5	54	>10	0.64/0.21	>10	>10	>10	>10
SYK	3.2	0.045	10	>10	>10	>10	>10
TAO2	1.1	0.71/0.46	>10	>10	>10	7.1/2.8	>10
TEC	ND	>10/ND	0.55/0.079	>10/ND	>10/ND	>10/ND	>10/ND
YES	31	1.1	0.0032	>10	>10	>10	>10
ZAK	19	10	0.1445	>10	5.4	0.103	>10

Six reference kinase inhibitors were profiled against two cell lines, HL60's and PC3's. A total of 209 kinases were evaluated and K_d's were determined. Only kinases inhibited by one of the five clinical compounds are illustrated here.

K_d or IC₅₀/K_d μM



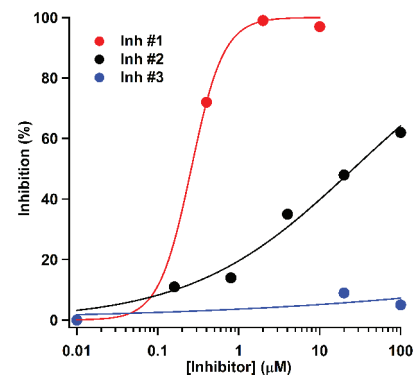
Lead Optimization

Native kinase profiling and inhibitor selectivity screen

KiNativ™ consistently demonstrates higher correlation with *in vivo* and cell-based assays compared to corresponding substrate-based assays with recombinant kinases. Several examples illustrated here highlight how KiNativ™ can identify the most efficacious inhibitors at the earliest stages of drug discovery.

	Recombinant IC ₅₀ (μM)	KiNativ™ IC ₅₀ (μM)	Cellular IKK-β phosphorylation EC ₅₀ (μM)
Inh. #1	0.08	0.8	0.33
Inh. #2	0.15	43	27
Inh. #3	0.34	>100	>100

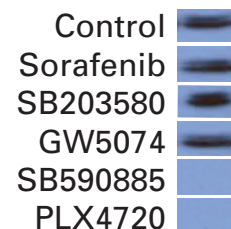
Inhibition of IKK-β phosphorylation



Three structural classes of TAK1 inhibitors were evaluated using a recombinant kinase assay, KiNativ™, and a cellular phosphorylation assay. The recombinant assay shows potent activity for all three inhibitors, whereas KiNativ™ accurately identifies compound #1 as the only active inhibitor.

	Recombinant IC ₅₀ (μM)	KiNativ™ IC ₅₀ (μM)	A375 cell proliferation EC ₅₀ (μM)
Sorafenib	0.18	>15	4
SB203580	0.309	15	>10
GW5074	0.024	3.8	>10
SB590885	0.043	0.009	0.03
PLX4720	0.09	0.005	0.03

α-phospho-ERK



Several reference B-Raf inhibitors were evaluated by a recombinant V600E-B-Raf substrate-based assay, KiNativ™ (A375 cells), an A375 cell proliferation assay, and a cellular Erk phosphorylation assay (at 2 μM in A375 cells). GW5074 appears to be the most potent V600E-B-Raf inhibitor by the substrate-based assay but is a poor B-Raf inhibitor in KiNativ™ and does not inhibit A375 (V600E B-Raf) proliferation or Erk phosphorylation. Overall the KiNativ™ data correlates well with cellular data for all compounds tested.

	Abl 1/2			SRC		
	KiNativ™ IC ₅₀ (nM)	Ambit IC ₅₀ (nM)	In vivo potency EC ₅₀ (nM)	KiNativ™ IC ₅₀ (nM)	Ambit IC ₅₀ (nM)	In vivo potency EC ₅₀ (nM)
Gleevec	150	12	200*	>10,000	>10,000	ND
Dasatinib	3.4	0.53	1.5*	3.3	0.21	4.3**

Data for gleevec and dasatinib were compared relative to the reported *in vivo* potency in a cellular autophosphorylation assay. While both Ambit and KiNativ™ correlate with relative response, the absolute potency is more accurate with KiNativ™.

* Manley, PW; et. al., Biochim. Biophys. Acta, 2005 Dec 30; 1754(1-2):3-13

** Du, J; et. al., Nat. Biotechnol. 2009 Jan; 27(1):77-83

