KiNativ™ Application Note

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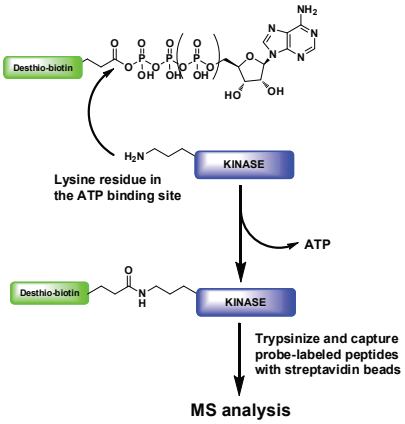
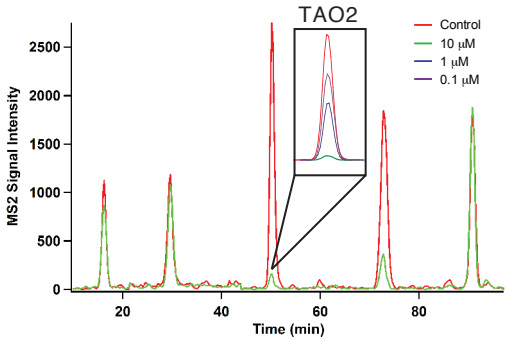
# Lead Optimization

## 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-MS$^2$ 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 |
| BTK | 86 | 0.91 | 0.013 | >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 | >10 | >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 | 0.1 | 5 | >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 I | 4.5 | >10 | 2.4 | >10 | 10 | >10 | >10 |
| p38d/g | 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 $IC_{50}$ /$K_d$ μM

| |
|---|
| <0.05 |
| 0.05-0.25 |
| 0.25-1 |
| 1-10x |
| >10 |

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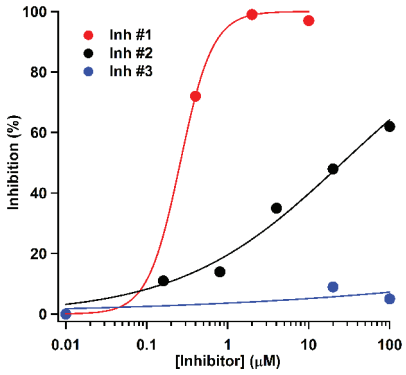
## Native kinase profiling and inhibitor selectivity screen

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 |

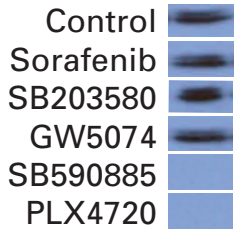
Inhibition of IKK-b phosphorylation




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 2μM 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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