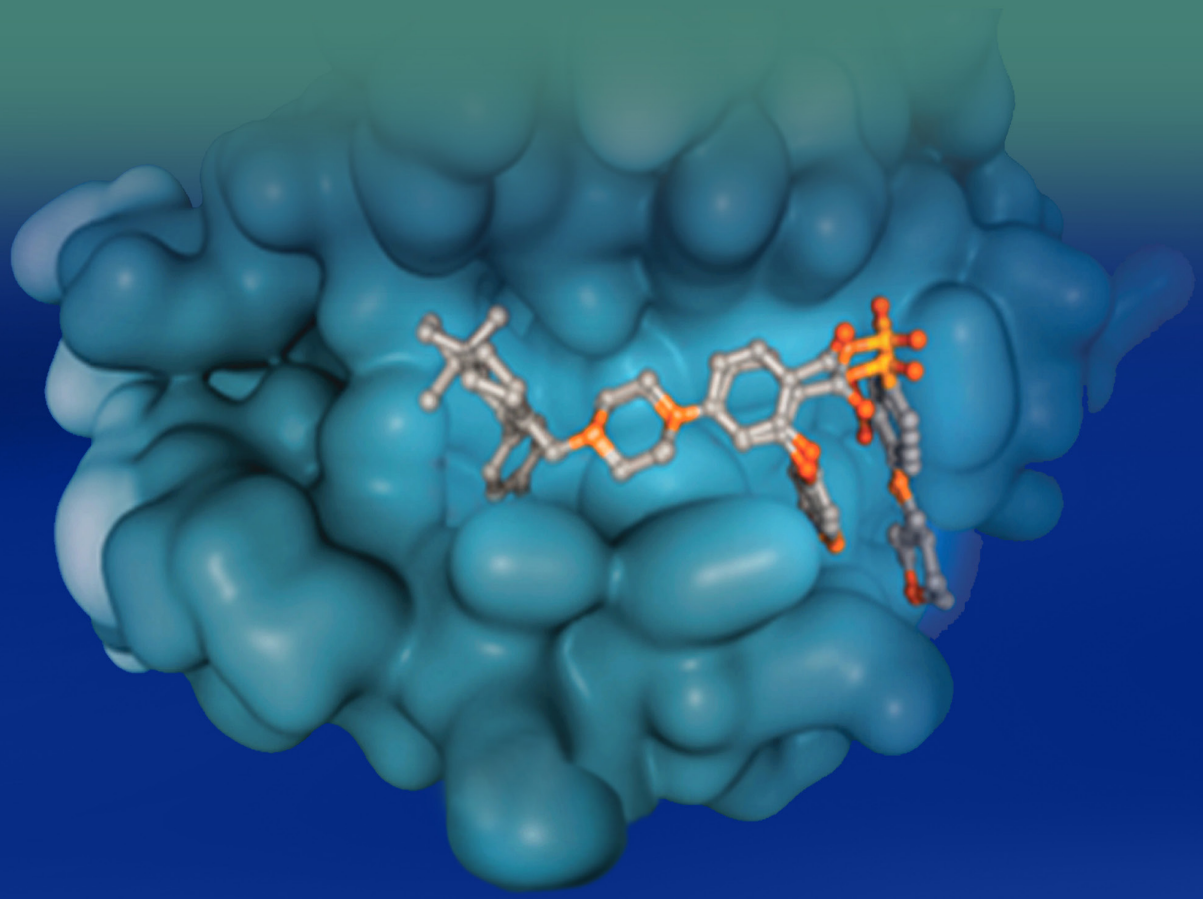
DiscoverX

KINASE REFERENCE SET

InCELL Pulse™ Target Engagement Assays

Validation Data for Diverse Kinases

- Cellular target engagement potency data for multiple kinase inhibitors
- Dose response curves for under-studied and well-studied kinases
- Quantitative EC_{50}s for kinases represented across the human kinom including common off-target kinases



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CELLULAR TARGET ENGAGEMENT ASSAYS FOR KINASE DRUG DISCOVERY

Monitoring cellular drug permeability and target engagement enables assessment of compound efficacy and confirmation of mode of action. InCELL Pulse target engagement assays provide the ability to confirm compound cell entry and target engagement while overcoming limitations of current target engagement assays. These simple binding assays require no target-specific chemical tracer or antibody reagents and provide a convenient solution when functional assays are difficult or unavailable. Additionally, these assays are designed for screening inhibitors, validating hits identified in biochemical assays, measuring cellular EC_{50} values, and ranking compounds – all in the cellular environment.

InCELL Pulse has been successfully applied to rapidly measure quantitative cellular target engagement potency values for inhibitors of diverse protein classes, including kinases, methyltransferases, and hydrolases. This reference set booklet demonstrates the broad utility for InCELL Pulse across the human kinome, where we have validated assays for kinases from seven of the eight phylogenetic groups. Kinase inhibitors often potently inhibit several off-target kinases in biochemical assays. Unfortunately, the relevance of these off-target interactions in a cellular setting is difficult to assess due to a lack of functional assays for diverse kinases. For this same reason, the validation of new kinase targets for drug discovery has been limited as well. Here we demonstrate the utility of InCELL Pulse for measuring cellular target engagement for inhibitors of diverse kinases, not only well-studied kinases, but also under-studied kinases and common off-target kinases identified in biochemical inhibitor selectivity screens.

InCELL Pulse ASSAY PRINCIPLE

InCELL Pulse cellular target engagement assays are based on a novel cellular application of the Eurofins DiscoverX Enzyme Fragment Complementation (EFC) technology to detect compound binding based on protein thermal stabilization. This technology utilizes β-galactosidase split into two inactive fragments, an enhanced ProLabel® (ePL) peptide (42 amino acids) and an enzyme acceptor (EA), that associate to form a fully active β-galactosidase enzyme. In the InCELL Pulse assays, the target is fused to ePL and expressed in the selected cell background. The fusion is detected with high sensitivity through the addition of EA and substrate in the presence of a cell lysis buffer.

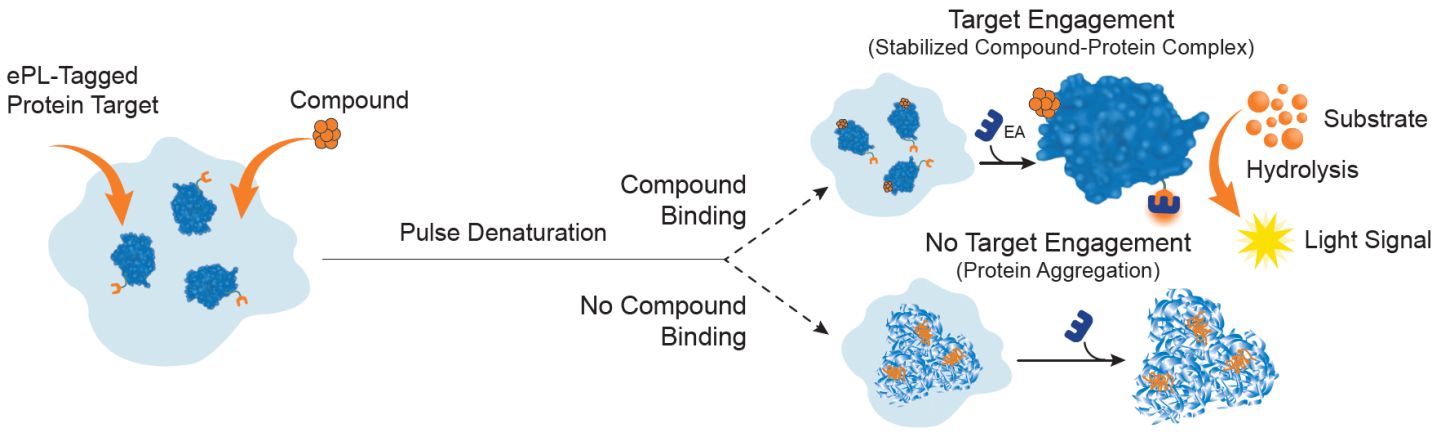
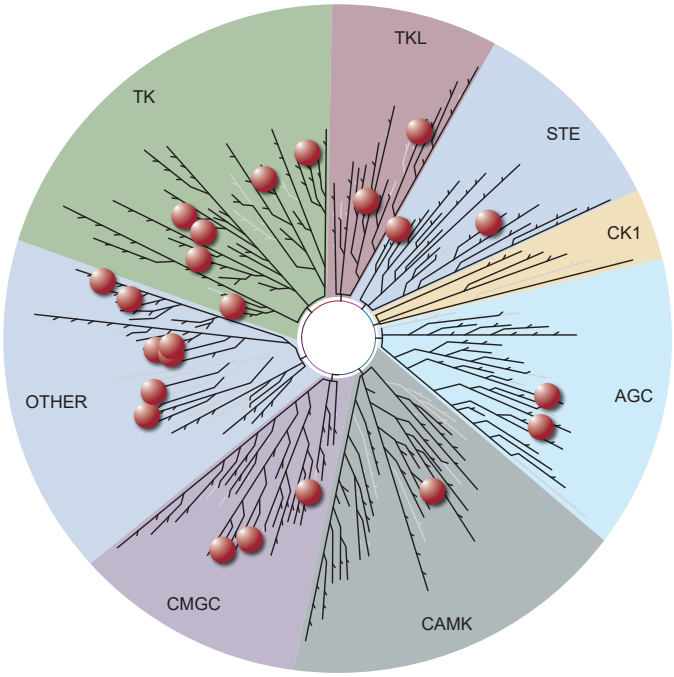


Figure 1. Cells expressing a protein of interest fused to the ePL tag are treated with test compound and then subjected to elevated temperatures during a pulse denaturation step. Compound binding protects the target protein from thermal denaturation, which enhances complementation between EA and ePL and increases the chemiluminescent signal measured using the EFC-based detection system. In the absence of compound binding, the target protein forms denatured aggregates that poorly complement with EA, which results in a low chemiluminescent signal.

BALANCED KINASE REPRESENTATION ACROSS DENDROGRAM



UNDER-STUDIED AND WELL-STUDIED KINASES REPRESENTED

Kinase Target		Class
ABL1	FAK	Tyr Kinase
ABL1(T315I)	IGF1R	
ACVR1	JAK2(JH1)	
BTK	KIT	
AAK1	GAK	Ser/Thr Kinase
AKT1	HASPIN	
AURKA	MEK1	
BRAF	p38-alpha	
BUB1	PAK4	
CAMK2A	PKAC-alpha	
CSNK2A2	PLK1	
ERK1	SRPK1	
VPS34		Lipid Kinase

Assays for all kinases listed in this Reference Set booklet are available for custom development. Please contact: DiscoverXCustomDevelopment@discovery.eurofinsus.com.

To learn more about the InCELL target engagement platform, visit discoverx.com/incell

Contact information at discoverx.com/support/

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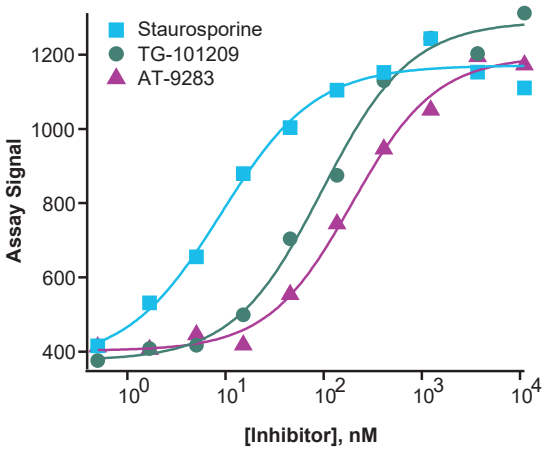
VALIDATION DATA FOR DIVERSE KINASES

AAK1 Ser/Thr Kinase

AP2 associated kinase 1: Regulates endocytosis. A potential target for treating neuropathic pain. Common off-target kinase identified in selectivity screens of diverse inhibitors.

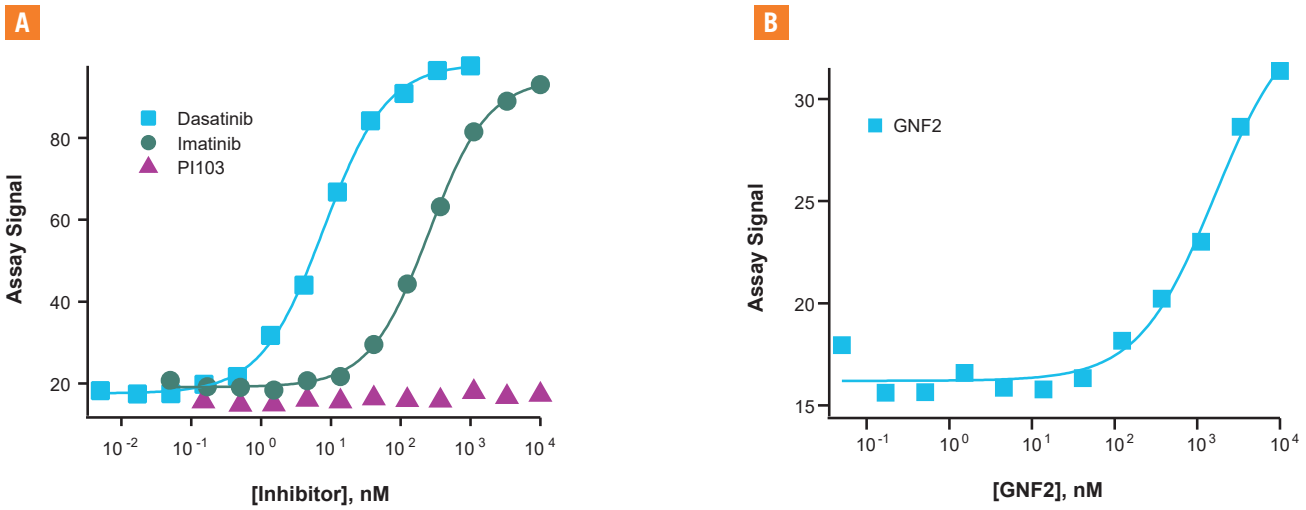


Inhibitor	EC_{50} (nM)	Assay Window
Staurosporine	9.0	2.6
TG-101209	96	3.0
AT-9283	190	**3.0**

Figure 2. Functional performance: AAK1 cellular target-engagement dose-response curves for diverse inhibitors with known biochemical activity. InCELL Pulse rank order potencies in good agreement with biochemical data.

ABL1 Tyr Kinase

ABL proto-oncogene 1, non-receptor tyrosine kinase: BCR-ABL fusions are established drivers in chronic myelogenous leukemia (CML). ABL1 inhibitors are effective CML therapies. Common off-target kinase identified in selectivity screens of diverse inhibitors.

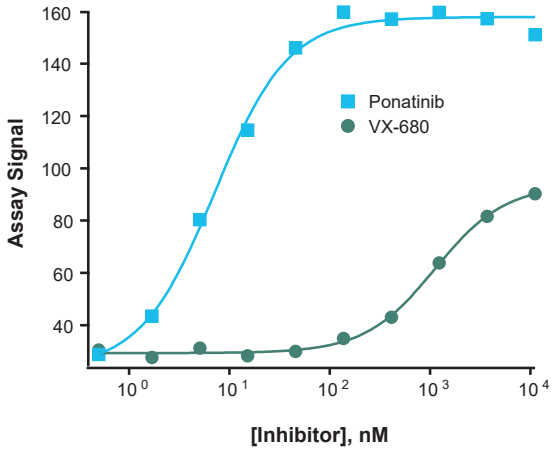


Inhibitor	Inhibitor Type	EC_{50} (nM)	Assay Window
Dasatinib	I	8.0	5.4
Imatinib	II	290	4.8
GNF2	Allosteric	2,300	2.3
PI103	Lipid Kinase Inhibitor	>10,000	n/a

Figure 3. Functional performance: ABL1 cellular target-engagement dose-response curves for Type I, Type II, and allosteric inhibitors. A. The Type I and Type II inhibitors dasatinib and imatinib, respectively, show the correct rank-order potencies. The lipid kinase inhibitor PI103 was included as a negative control. B. The allosteric inhibitor GNF2, which targets the myristate binding site in the C-terminal kinase lobe, is detected using the ABL1 InCELL Pulse assay.

ABL1(T315I) Tyr Kinase

ABL proto-oncogene 1, non-receptor tyrosine kinase, T315I mutant: Drug-resistant "gatekeeper" mutant of ABL1 conferring kinase inhibitor resistance in acute myelogenous leukemia. Common off-target kinase identified in selectivity screens of diverse inhibitors.

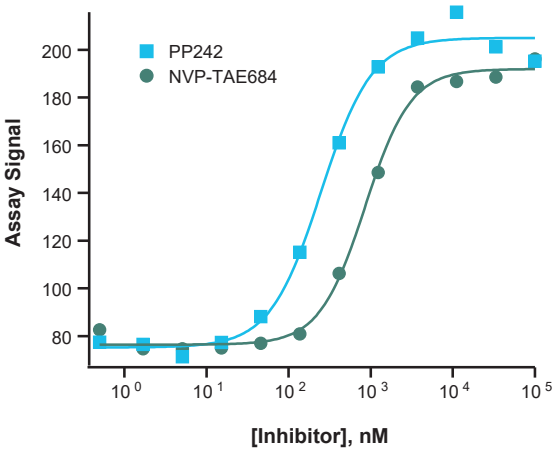


Inhibitor	EC_{50} (nM)	Assay Window
Ponatinib	7.1	5.1
VX-680	1,100	3.2

Figure 4. Functional performance: ABL1(T315I) cellular target-engagement dose-response curves for diverse inhibitors known to overcome cellular resistance conferred by the T315I gatekeeper mutation. InCELL Pulse rank order potencies agree with known values, with ponatinib being highly potent against this drug-resistant ABL1 mutant.

ACVR1 Tyr Kinase

Activin A receptor, type I: Also known as ALK2. Transmembrane receptor kinase related to the bone morphogenetic protein (BMP) receptor kinase family. Rare activating mutations cause bone disease and childhood brain tumors.

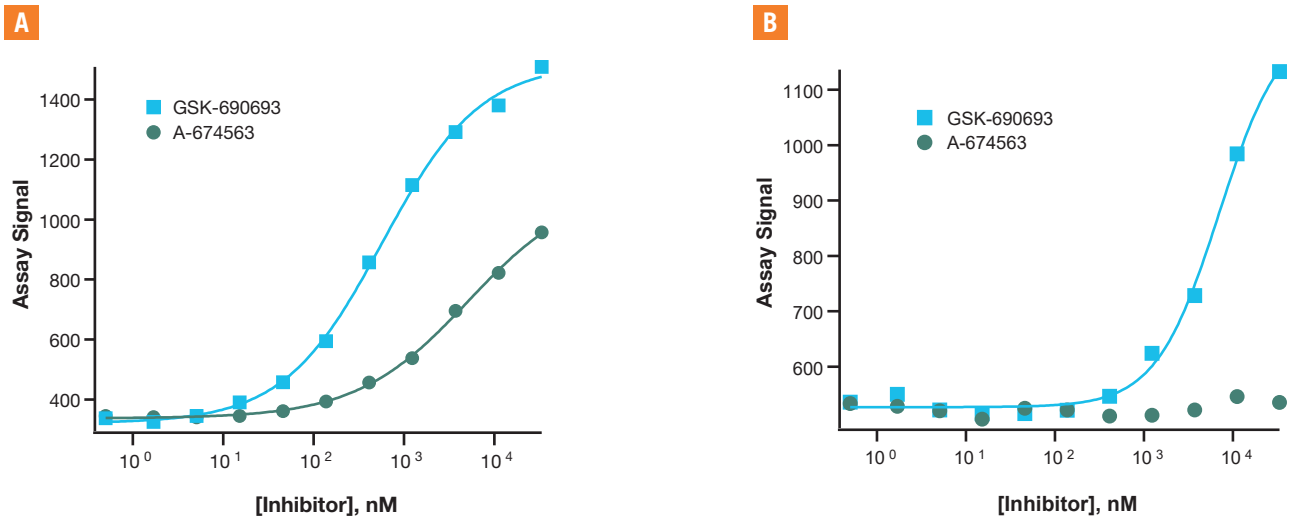


Inhibitor	EC_{50} (nM)	Assay Window
PP242	250	2.7
NVP-TAE684	850	2.5

Figure 5. Functional performance: ACVR1 cellular target-engagement dose-response curves for diverse inhibitors with known biochemical activity. InCELL Pulse rank order potencies in good agreement with biochemical data.

AKT1 Ser/Thr Kinase

v-akt murine thymoma viral oncogene homolog 1: Oncogenic kinase in the PI3K/AKT pathway implicated in diverse disease indications.

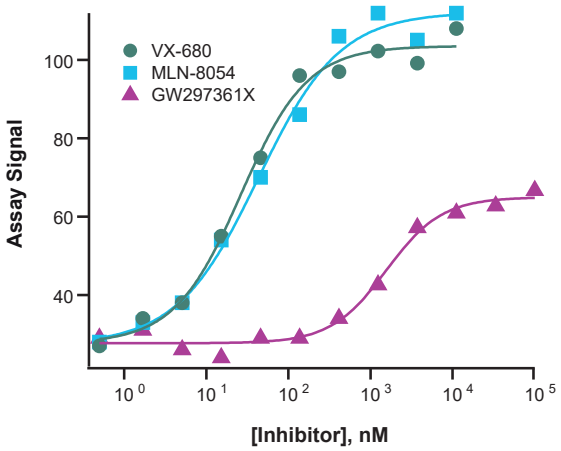


Inhibitor	AKT1 Activation State	EC_{50} (nM)	Assay Window
GSK-690693	Activated	570	4.7
A-674563	Activated	5,100	3.3
GSK-690693	Un-activated	7,000	2.3
A-674563	Un-activated	>30,000	n/a

Figure 6. Functional performance: AKT1 cellular target-engagement dose-response curves for dedicated AKT1 inhibitors. A. AKT1 was activated by replacing the hydrophobic motif (HM) with a peptide that docks in the PIF pocket (PIFtide). Potent activity is measured for both AKT1 inhibitors. B. Un-activated AKT1 construct harboring a wild-type HM motif. The un-activated form shows greatly reduced affinity for both AKT1 inhibitors tested.

AURKA Ser/Thr Kinase

Aurora kinase A: Mitotic kinase target for oncology indications, but inhibition causes toxic side effects. Undesirable off-target for kinase drug discovery programs. Common off-target kinase identified in selectivity screens of diverse inhibitors.

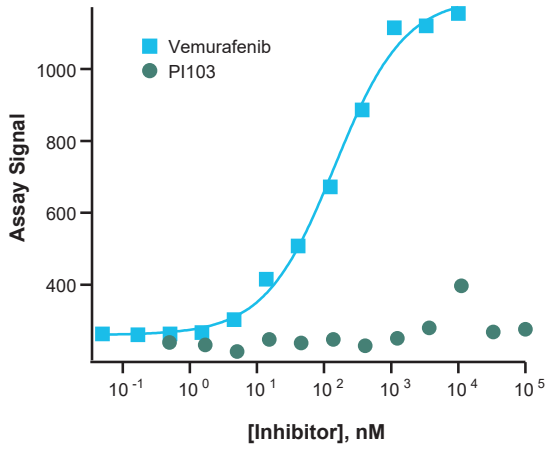


Inhibitor	EC_{50} (nM)	Assay Window
VX-680	26	3.8
MLN-8054	43	4.1
GW297361X	1,600	2.3

Figure 7. Functional performance: AURKA cellular target-engagement dose-response curves for dedicated AURKA inhibitors (VX-680, MLN-8054) and one inhibitor shown to inhibit AURKA in biochemical screens (GW297361X). InCELL Pulse rank order potencies agree with published data.

BRAF Ser/Thr Kinase

B-Raf proto-oncogene, serine/threonine kinase: Key signaling kinase in the RAS/RAF/MEK/ERK pathway. The BRAF(V600E) mutant is a driver in melanoma and other oncology indications.

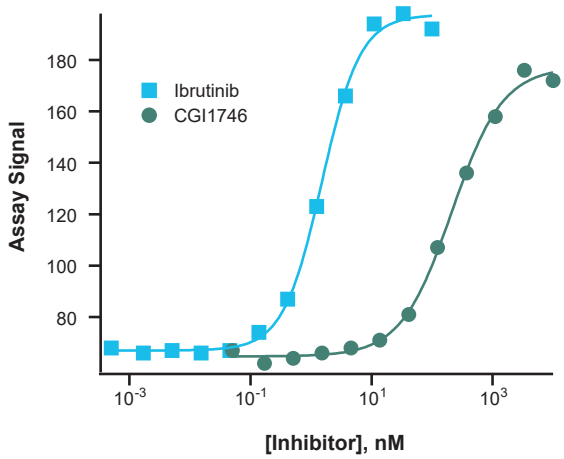


Inhibitor	EC_{50} (nM)	Assay Window
Vemurafenib	140	4.6
PI103	>100,000	n/a

Figure 8. Functional performance: BRAF cellular target-engagement dose-response curves for the FDA-approved inhibitor vemurafenib and the lipid kinase inhibitor PI103, which serves as a negative control. InCELL Pulse rank order potencies agree with published data.

BTK Tyr Kinase

Bruton tyrosine kinase: BTK plays a critical role in B cell development and is a validated drug target for B cell cancers. BTK mutations are implicated in immunodeficiency disease. Common off-target kinase identified in selectivity screens of diverse inhibitors.

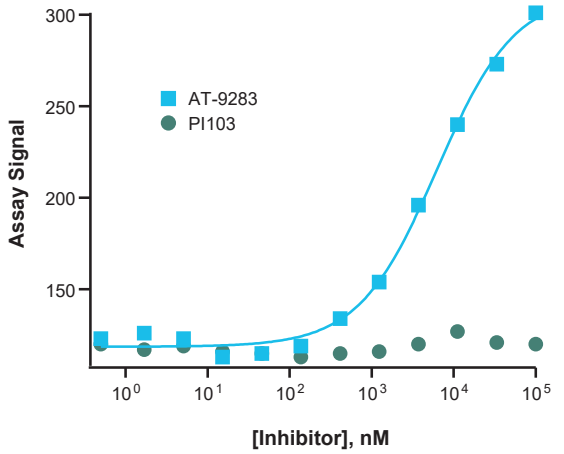


Inhibitor	Inhibitor Type	EC_{50} (nM)	Assay Window
Ibrutinib	Irreversible	1.5	3.0
CGI1746	Reversible	210	2.7

Figure 9. Functional performance: BTK cellular target-engagement dose-response curves for irreversible (ibrutinib) and reversible (CGI1746) inhibitors. Ibrutinib is FDA-approved for the treatment of B cell cancers. Both inhibitor types show potent activity in the BTK InCELL Pulse assay.

BUB1 Ser/Thr Kinase

BUB1 mitotic checkpoint serine/threonine kinase: Mitotic checkpoint kinase also believed to regulate the TGF-β signaling pathway. Despite its potential disease-relevance, BUB1 is an under-studied kinase due to a lack of available biochemical and cellular assays.

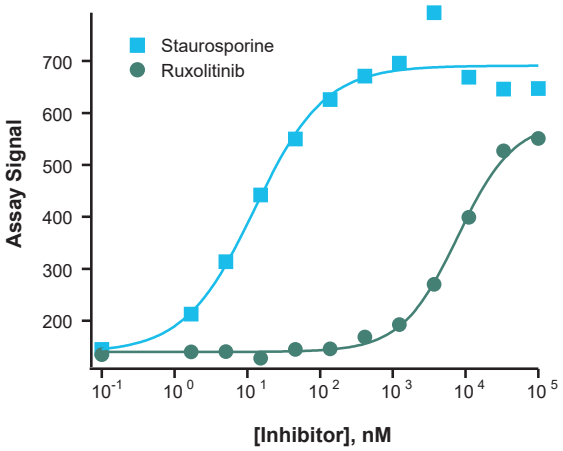


Inhibitor	EC_{50} (nM)	Assay Window
AT-9283	6,200	2.6
PI103	>100,000	n/a

Figure 10. Functional performance: BUB1 cellular target-engagement dose-response curves for AT-9283, which has BUB1 activity in a KINOME*scan*® biochemical assay. The lipid kinase inhibitor PI103 serves as a negative control.

CAMK2A Ser/Thr Kinase

Calcium/calmodulin dependent protein kinase II α: Associated with Alzheimer's disease and heart arrhythmia.

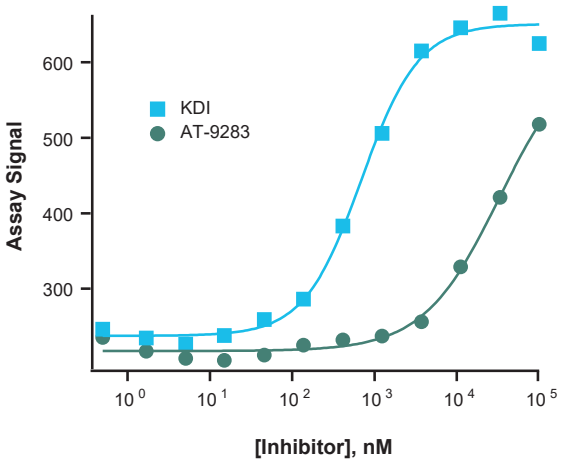


Inhibitor	EC_{50} (nM)	Assay Window
Staurosporine	13	5.0
Ruxolitinib	7,800	4.2

Figure 11. Functional performance: CAMK2A cellular target-engagement dose-response curves for two inhibitors identified in KINOME*scan*® biochemical screens: the potent JAK2 inhibitor ruxolitinib and the pan-kinase inhibitor staurosporine. InCELL Pulse rank order potencies agree with the biochemical data.

CSNK2A2 Ser/Thr Kinase

Casein kinase 2 α2: Proposed to have roles in diverse cellular processes related to disease and to be activated by the Wnt signaling pathway.

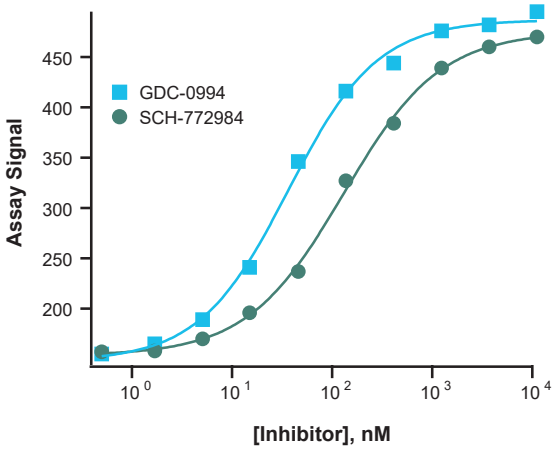


Inhibitor	EC_{50} (nM)	Assay Window
KDI	690	2.7
AT-9283	33,000	2.9

Figure 12. Functional performance: CSNK2A2 cellular target-engagement dose-response curves for Keratinocyte Differentiation Inducer (KD1) and AT-9283, which both have activity in KINOME*scan* biochemical CSNK2A2 assays. InCELL Pulse rank order potencies agree with the biochemical data.

ERK1 Ser/Thr Kinase

Mitogen-activated protein kinase 1: Key signaling kinase in the RAS/RAF/MEK/ERK pathway. Oncology target in tumors resistant to BRAF and MEK inhibitors.

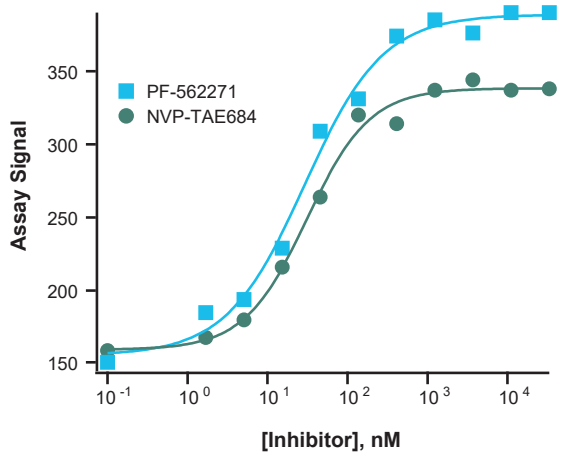


Inhibitor	EC_{50} (nM)	Assay Window
GDC-0994	36	3.3
SCH-772984	130	3.1

Figure 13. Functional performance: ERK1 cellular target-engagement dose-response curves for two well-studied potent inhibitors. InCELL Pulse rank order potencies agree with published biochemical data.

FAK Tyr Kinase

Protein tyrosine kinase 2: In preclinical tumor models, inhibition of FAK signaling synergizes with immuno-oncology agents. FAK inhibitors are being pursued clinically for oncology indications in combination with immuno-oncology agents. Common off-target kinase identified in selectivity screens of diverse inhibitors.

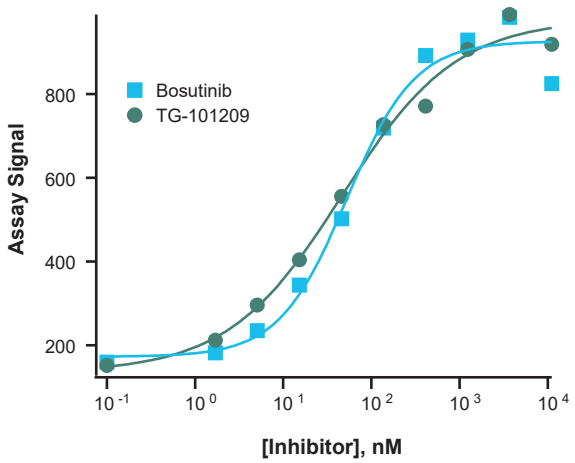


Inhibitor	EC_{50} (nM)	Assay Window
PF-562271	29	2.5
NVP-TAE684	31	2.1

Figure 14. Functional performance: FAK cellular target-engagement dose-response curves for a dedicated FAK inhibitor (PF-562271) and an inhibitor with potent off-target FAK activity (NVP-TAE684). InCELL Pulse potency values are similar for these inhibitors, which agrees with the biochemical potency data.

GAK Ser/Thr Kinase

Cyclin G associated kinase: Under-studied kinase believed to function downstream of p53. Common off-target kinase identified in selectivity screens of diverse inhibitors.

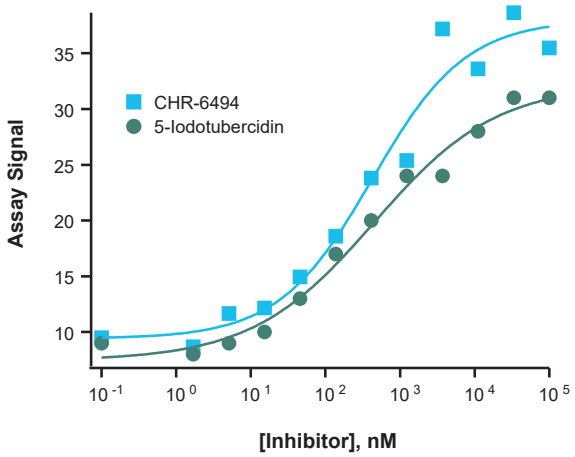


Inhibitor	EC_{50} (nM)	Assay Window
Bosutinib	46	7.2
TG-101209	52	5.4

Figure 15. Functional performance: GAK cellular target-engagement dose-response curves for inhibitors with known biochemical activity in a KINOME*scan*® GAK assay. InCELL Pulse potency values are similar for these inhibitors, which agrees with the biochemical potency data.

HASPIN Ser/Thr Kinase

Germ cell associated 2, haspin: Mitotic and meiotic kinase that is a potential oncology target. Phosphorylates histone H3 at Thr3, which is required for mitosis progression. Inhibitors arrest cells in G2/M. Also reported to regulate the meiotic kinase Aurora C.

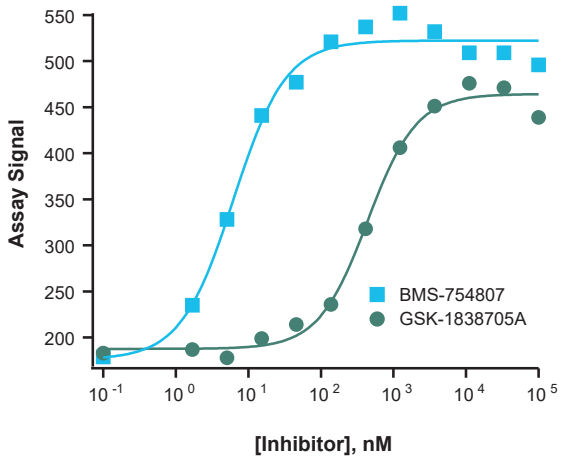


Inhibitor	EC_{50} (nM)	Assay Window
CHR-6494	420	4.0
5-Iodotubercidin	450	4.3

Figure 16. Functional performance: HASPIN cellular target-engagement dose-response curves for a dedicated HASPIN inhibitor (CHR-6494) and a pan-kinase inhibitor with potent off-target HASPIN activity in KINOME*scan* assays (5-iodotubercidin). Biochemical HASPIN potency data using a common assay format are not available for these inhibitors.

IGF1R Tyr Kinase

Insulin like growth factor 1 receptor: Potential target for diverse cancer types. Clinical inhibitors have had activity in subsets of tumors and patient populations. IGF1R inhibitors often potently cross-react with the insulin receptor tyrosine kinase resulting in metabolic toxicity.

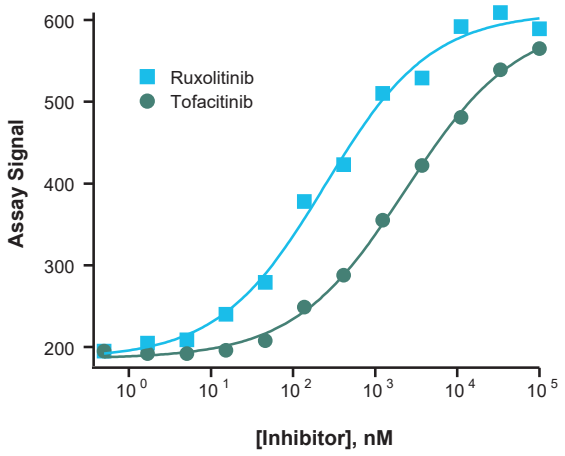


Inhibitor	EC_{50} (nM)	Assay Window
BMS-754807	6.2	3.0
GSK-1838705A	440	2.5

Figure 17. Functional performance: Cellular target-engagement dose-response curves for two dedicated IGF1R inhibitors, BMS-754807 and GSK-1838705A. InCELL Pulse rank order potencies agree with published cell potency values measuring inhibition of autophosphorylation.

JAK2(JH1) Tyr Kinase

Janus kinase 2: JAK2 contains two kinase domains. JH1 is the catalytically active kinase domain and is used in this assay. The JAK2(V617F) mutation drives several myeloproliferative disorders that are treated with JAK2 inhibitors. Common off-target kinase identified in selectivity screens of diverse inhibitors.

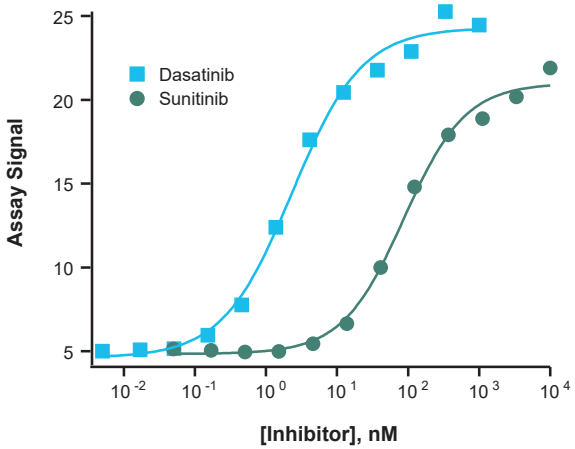


Inhibitor	EC_{50} (nM)	Assay Window
Ruxolitinib	240	3.3
Tofacitinib	2,300	3.2

Figure 18. Functional performance: Cellular target-engagement dose-response curves for two dedicated JAK family inhibitors, tofacitinib (JAK3-selective) and ruxolitinib (JAK2-selective). InCELL Pulse JAK2(JH1) domain rank order potencies agree with published data.

KIT Tyr Kinase

KIT proto-oncogene receptor tyrosine kinase: Activating mutations in KIT drive diverse cancers and mastocytosis. Dual inhibition of KIT and the closely related kinase FLT3 causes neutropenia. Common off-target kinase identified in selectivity screens of diverse inhibitors.

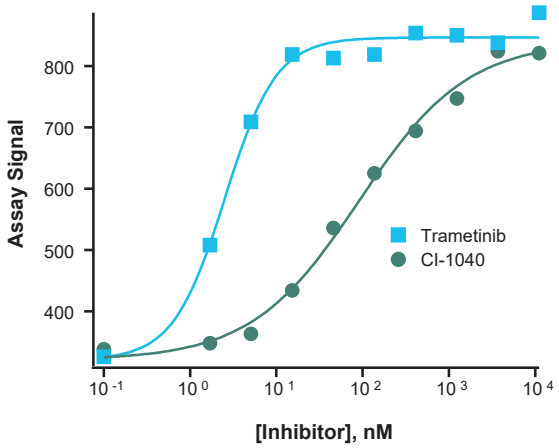


Inhibitor	EC_{50} (nM)	Assay Window
Dasatinib	2.3	5.2
Sunitinib	87	4.3

Figure 19. Functional performance: Cellular target-engagement dose-response curves for two well-validated KIT inhibitors, dasatinib, and sunitinib. InCELL Pulse rank order potencies agree with data from a proliferation assay using a KIT-dependent Ba/F3 cell line, while the inhibitor potencies can be more similar in KIT autophosphorylation assays.

MEK1 Ser/Thr Kinase

Mitogen-activated protein kinase kinase 1: Clinically validated oncology target in the RAS/RAF/MEK/ERK pathway. Non-ATP competitive MEK inhibitors are used in combination with BRAF inhibitors in BRAF(V600E)-driven cancers. Common off-target kinase identified in selectivity screens of diverse inhibitors.

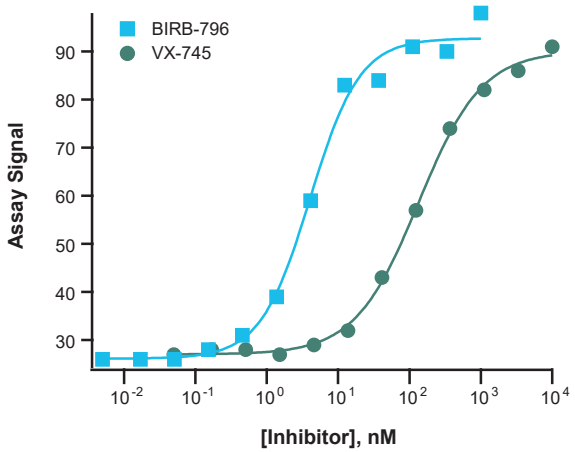


Inhibitor	EC_{50} (nM)	Assay Window
Trametinib	2.5	2.6
CI-1040	91	2.6

Figure 20. Functional performance: Cellular target-engagement dose-response curves for two dedicated non-ATP competitive MEK1 inhibitors. InCELL Pulse rank order potencies agree with published data, with the FDA-approved inhibitor trametinib being more potent than the preclinical compound CI-1040.

p38-alpha Ser/Thr Kinase

Mitogen-activated protein kinase 14: Regulates cytokine release and has historically been a promising target for inflammatory disease. Recent data suggest oncology applications as well.

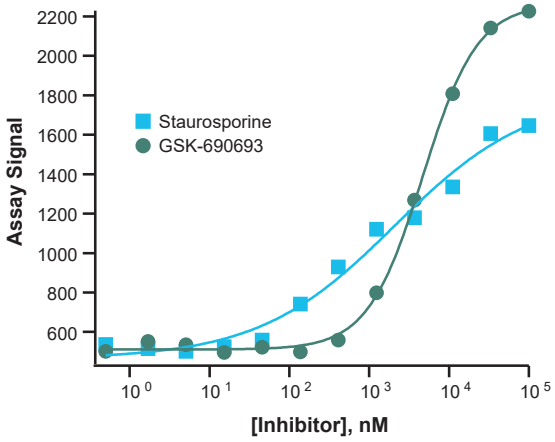


Inhibitor	EC_{50} (nM)	Assay Window
BIRB-796	4.1	3.6
VX-745	130	3.3

Figure 21. Functional performance: Cellular target-engagement dose-response curves for Type I (VX-745) and Type II (BIRB-796) dedicated p38-alpha inhibitors. InCELL Pulse rank order potencies agree with published biochemical data.

PAK4 Ser/Thr Kinase

p21 (RAC1) activated kinase 4: Essential roles in embryonic development and overexpression is associated with some cancers.

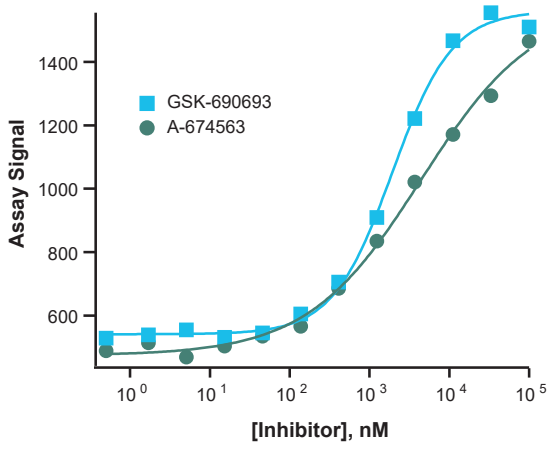


Inhibitor	EC_{50} (nM)	Assay Window
Staurosporine	2,100	3.9
GSK-690693	4,700	4.4

Figure 22. Functional performance: Cellular target-engagement dose-response curves for two PAK4 inhibitors identified in KINOME*scan*® biochemical screens, GSK-690693 (a dedicated AKT1 inhibitor) and the pan-kinase inhibitor staurosporine. InCELL Pulse rank order potencies agree with KINOME*scan* data.

PKAC-alpha Ser/Thr Kinase

Protein kinase cAMP-activated catalytic subunit α: Metabolic regulatory kinase with cAMP-dependent activity.

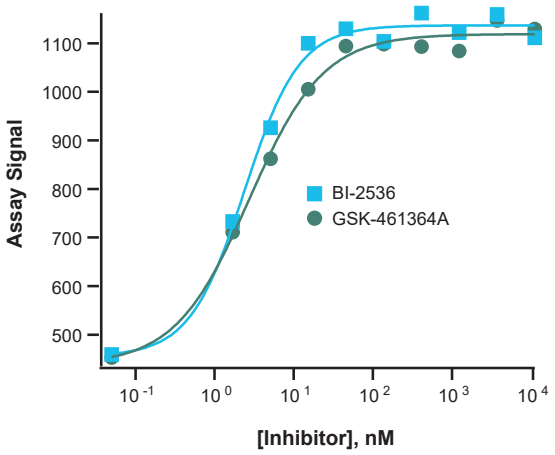


Inhibitor	EC_{50} (nM)	Assay Window
GSK-690693	1,900	2.9
A-674563	4,400	3.3

Figure 23. Functional performance: Cellular target-engagement dose-response curves for two PKAC-alpha inhibitors identified in KINOME*scan*® biochemical screens, GSK-690693 and A-674563, which are both dedicated inhibitors of the related AGC family kinase AKT1. InCELL Pulse rank order potencies agree with KINOME*scan* data.

PLK1 Ser/Thr Kinase

Polo like kinase 1: Mitotic kinase pursued for cancer indications. Inhibition results in hematopoietic toxicities including neutropenia and thrombocytopenia. Common off-target kinase identified in selectivity screens of diverse inhibitors.

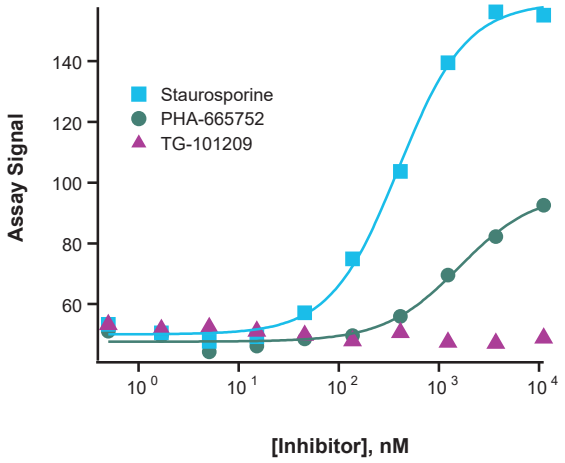


Inhibitor	EC_{50} (nM)	Assay Window
BI-2536	2.4	2.5
GSK-461364A	2.7	2.6

Figure 24. Functional performance: Cellular target-engagement dose-response curves for two dedicated equipotent PLK1 inhibitors, BI-2536 and GSK-461364A. InCELL Pulse measures equipotent cellular activity for these inhibitors, as expected.

SRPK1 Ser/Thr Kinase

SRSF protein kinase 1: Regulates pre-mRNA splicing by controlling localization of splicing factors. SRPK1 inhibitors manipulate VEGF-A splicing to favor the anti-angiogenic form, suggesting applications to wet macular degeneration. Common off-target kinase identified in selectivity screens of diverse inhibitors.

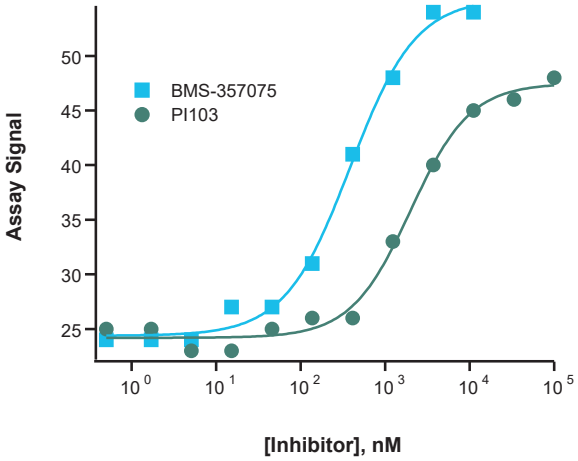


Inhibitor	EC_{50} (nM)	Assay Window
Staurosporine	400	3.2
PHA-665752	1,600	2.0
TG-101209	>10,000	n/a

Figure 25. Functional performance: Cellular target-engagement dose-response curves for one potent (staurosporine) and two less potent (PHA-665752 and TG-101209) SRPK1 inhibitors identified in KINOME*scan*® biochemical screens. InCELL Pulse confirms cellular target engagement for staurosporine and PHA-665752.

VPS34 Lipid Kinase

Phosphatidylinositol 3-kinase catalytic subunit type 3: Regulates autophagy. Inhibitors block autophagy and synergize with mTOR inhibitors when tested in renal tumor cell lines.



Inhibitor	EC_{50} (nM)	Assay Window
BMS-357075	390	2.3
PI103	2,000	2.0

Figure 26. Functional performance: Cellular target-engagement dose-response curves for two VPS34 inhibitors identified in KINOME*scan* biochemical screens, the dedicated CDK inhibitor BMS-357075 and the pan-lipid kinase inhibitor PI103. InCELL Pulse rank order potencies agree with KINOME*scan* data.