A Flexible Workflow for Automated Bioluminescent Kinase Selectivity Profiling

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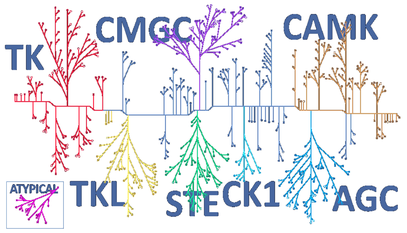
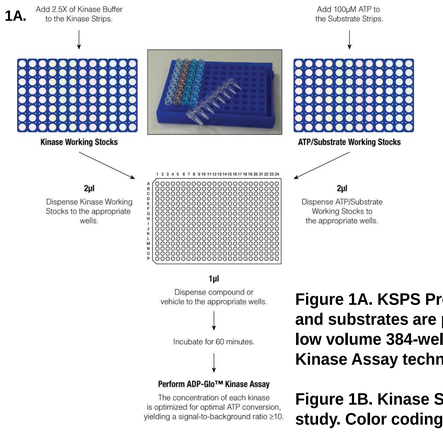
1. Introduction

Kinase profiling is a necessary process to confirm inhibitor selectivity and assess off-target activity. The arduous task of optimizing kinase reactions and maintaining a library of enzymes often results in the outsourcing of kinase profiling activities.



We aim to show how ready-to-use Kinase Selectivity Profiling Systems (KSPS) combined with easy-to-use instrumentation and automated data analysis supports a streamlined workflow to enable quick and efficient in-house kinase profiling.

2. Kinase Profiling Protocol Overview



1B.

TK-2	TK-4	CMGC-1	General Panel		
ABL1	c-MER	ERK2	FGFR1	CDK2/CyclinE1	AKT1
BRK	FGFR1	GSK3b	JAK3	GSK3b	PKCa
BTK	FGFR2	JNK1	LCK	p38a	ROCK1
CSK	FGFR4	JNK3	SYK	AMPK A1/B1/G2	Aurora A
FYN A	FLT1	p38a	MINK1	CAMK4	CK2a1
LCK	FMS	p38b	PAK1/cdc42	CHK1	IKKb
LYN B	MET	p38d	IRAK4	DAPK1	CK1a1
SRC	RET	p38g	TAK1-TAB1	MAPKAPK2	CK1g1

Figure 1A. KSPS Protocol: Pre-dispensed strips of kinases and substrates are prepared and reactions assembled in a low volume 384-well assay plate (Corning 4512). ADP-Glo™ Kinase Assay technology is used to quantify kinase activity.

Figure 1B. Kinase Selectivity Profiling Systems used in this study. Color coding corresponds to different kinase families.

3. Incorporating Automation Throughout the Profiling Workflow

Figure 2. Reaction assembly, detection, and data analysis are automatically conducted according to user-input parameters for single or dose-response testing of up to two kinase strips per plate. Automated data analysis tools compute % kinase activity for up to twenty compounds (single-dose inhibition profile), or IC_{50} values for ten-point titration of up to two compounds (dose-response profile).

4. An Overview of the Automation Process for Single-Point and Dose-Response Profiling Experiments

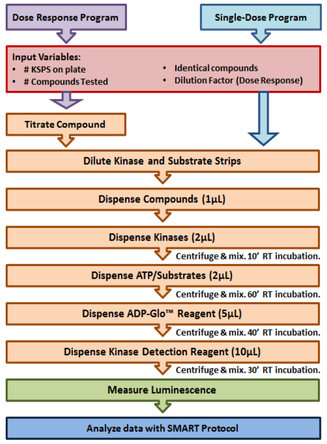


Figure 3. The user selects the desired profiling protocol to run and reagents are added to the bed of the PIPETMAX®. The PIPETMAX® performs all liquid handling steps as shown here. Centrifugation, mixing, and detection are performed off-line, while room temperature reaction incubations are performed on the bed of the instrument.

Input variables within the TRILUTION® micro run software* determine volume ratios for compound titration (if applicable), dictate the number of kinase and substrate strips to prepare per run, and guide reaction setup in the assay plate. Reactions are assembled according to preset templates that are later used for detection and data analysis.

*Protocols are available upon request.

5. A Single-Dose Profile Against the General Panel Confirms Selectivity and Reveals Off-Target Activity

		Bosutinib	Imatinib	Kenpaullone	Ponatinib	SU6656	Sunitinib	Tofacitinib	VX-702	Staurosporine
GP-1	FGFR1	39	82	77	4	29	22	71	81	2
	JAK3	58	98	95	7	82	68	0	95	-1
	LCK	0	69	75	0	57	33	99	107	1
	SYK	15	85	39	97	60	55	87	89	-2
	MINK1	4	101	43	65	100	42	109	85	1
	PAK1/CDC42	69	103	98	125	108	104	103	92	32
	IRAK4	42	95	97	99	54	28	106	106	2
	TAK1-TAB1	90	102	89	35	101	30	109	108	5
GP-2	CDK2/CyclinE1	94	101	38	86	98	93	93	95	2
	GSK3β	86	90	5	88	88	74	86	86	2
	p38α	48	92	97	1	95	95	80	0	60
	AMPK A1/B1/G2	53	103	82	82	21	7	77	88	1
	CAMK4	80	101	96	96	101	25	94	93	3
	CHK1	75	109	94	105	102	26	94	92	0
	DAPK1	102	102	101	101	105	73	100	98	4
	MAPKAPK2	85	87	85	89	85	84	87	88	26
GP-3	AKT1	99	99	100	93	97	88	103	101	0
	PKCα	78	102	101	94	92	85	62	78	1
	ROCK1	68	96	50	93	78	58	95	87	1
	Aurora A	100	103	101	105	40	89	82	107	4
	CK2α1	98	97	85	100	103	94	103	102	87
	IKKβ	94	95	96	60	88	89	86	83	27
	CK1α1	84	96	98	99	97	60	89	88	81
	CK1γ1	101	94	98	94	97	50	93	94	88

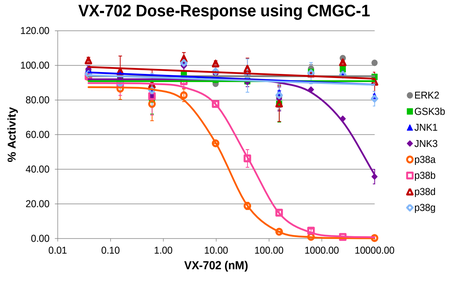
Table 1. KSPS General Panel results. Shown here is % kinase activity remaining following treatment with 1µM compound. Color coding represents:

>60% Activity
20-60% Activity
<20% Activity

We conducted secondary testing on Ponatinib and VX-702. VX-702 exhibits on-target inhibition of p38α. Further dose-response testing will confirm potency on p38α and like kinases of the CMGC-1 family.

Ponatinib shows expected on-target inhibition of the Abl-associated LCK and FGFR tyrosine kinases, but unexpected off-target inhibition of p38α. Dose-response experiments will verify potency for tyrosine kinases from KSPS: TK-2 and TK-4 and confirm off-target effects with KSPS: CMGC-1 kinases.

6. VX-702 Exhibits Potent Inhibition Against the Target p38α and Related Kinases



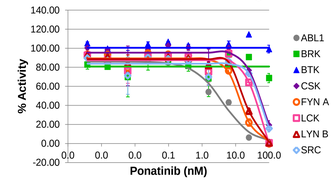
Kinase	IC_{50} Value
p38α	15nM
p38β	41nM

Figure 4. Dose response experiments were performed to evaluate VX-702 potency against the target p38α kinase and related kinases in the CMGC-1 family.

A serial 1:4 titration of VX-702 against KSPS: CMGC-1 kinases confirms potent inhibition of the target p38α kinase and closely related p38β kinase. Reactions are performed in duplicate.

6. Ponatinib Inhibition of the Target Abl1 Kinase and Related TK-2 and TK-4 Kinases Is Observed

5A. Ponatinib Dose-Response using TK-2



Kinase	IC_{50} Value
ABL1	4nM
LCK	29nM
FGFR1	9nM

Figure 5A. A serial 1:4 titration of Ponatinib confirms potent inhibition of the target Abl1 kinase and other related kinases from KSPS: TK-2.

5B. Ponatinib Dose-Response using TK-4

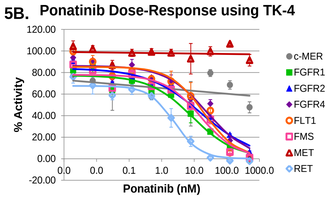
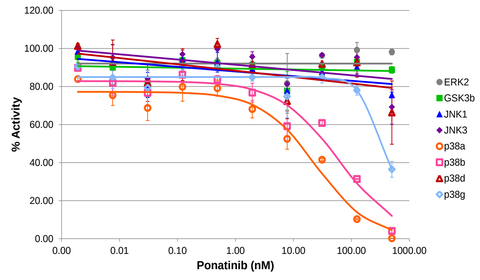


Figure 5B. Ponatinib inhibition of FGFR kinases from KSPS: TK-4 is confirmed.

8. Off-Target Ponatinib Inhibition of CMGC-1 Kinases Is Confirmed

Ponatinib Dose-Response using CMGC-1



Kinase	IC_{50} Value
p38α	24nM
p38β	60nM

Figure 6. A serial 1:4 titration of Ponatinib with KSPS: CMGC-1 confirms the initial hit on p38α from the single-dose profile and reveals potent inhibition of both p38α and p38β kinases.

9. Conclusions

Kinase Selectivity Profiling Systems come ready-to-use:

- Thaw strips and reagents, prepare ATP and reaction buffer working solutions, then place onto bed of PIPETMAX®.

Adaptive and reliable liquid handling with PIPETMAX® enhances KSPS ease-of-use:

- Consistent execution of liquid handling steps.
- On-the-fly adjustment of all liquid handling in accordance with experimental plate layout.
- Complete automation achieved from compound addition through detection reagent addition.

Efficient data analysis:

- The Discover SMART protocol export file minimizes time and effort to process data and quantify results.

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