ADP-Glo™ Kinase Profiling Systems for targeted and flexible kinase inhibitor profiling

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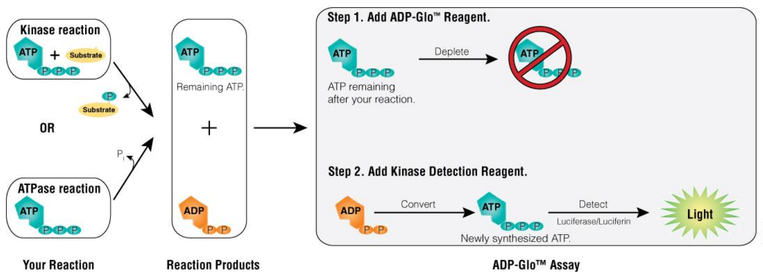
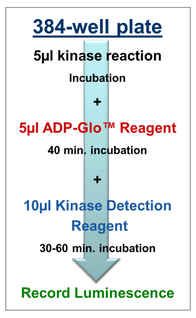
1. Abstract

Drug safety is of paramount importance in the pharmaceutical industry indicating that minimal side effects constitute a major requirement in drug development. Therefore, novel drug candidates need to be profiled against various liability targets, including a broad panel of kinases to provide a better understanding of off-target activities. Potentially, profiling can also identify new targets that may lead to novel therapeutic indications. A universal, robust and affordable technology is desirable to assess selectivity and potency of drug candidates against multiple classes of kinases. The luminescent ADP-Glo™ kinase assay is a universal platform that measures kinase activity by quantifying the amount of ADP produced during the enzymatic reaction. We have tested the utility of this platform with 174 optimized Kinase Enzyme Systems (KES) spanning different families of the human kinome. Here we present standardized Kinase Profiling Systems for simple kinase inhibitor profiling studies.The Kinase Profiling Systems are a set of kinases organized by families and presented in easy to use multi-well strips. Each strip contains eight enzymes each with their corresponding substrates, and standardized for optimal kinase activity for inhibitor profiling. Using the profiling strips we easily generated selectivity profiles, identifying compound promiscuity towards members of a single kinase subfamily or different subfamilies of the kinome. The ADP-Glo™ KES platforms now address the needs of basic kinase characterization, kinase screening, mode of action (MOA) studies and profiling in an affordable manner using one assay format.

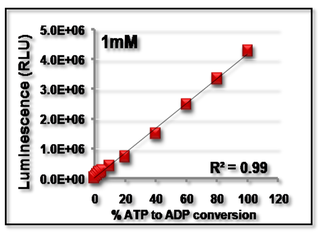
2. ADP-Glo™ is a positive detection Assay for product formation.

Assay concept, formats and Features





White Plates	Format 1:1:2 (µl)	
1,536-well	2.5/2.5/5	
384-well	5/5/10	10/10/20
96-well	25/25/50	50/50/100



- **Universal: Any kinase-substrate combination and ATPases.**
- **Wide dynamic range: High sensitivity at low % ATP to ADP conversion allows use of lower amount of enzyme.**
- **Broad range of [ATP]: (µM to mM) allows distinction between ATP competitive and non competitive inhibitors.**
- **Robust Assay: Homogenous with Z' higher than 0.7.**

3. Promega validated kinase panel covers the human Kinome.

Kinase Enzyme System (KES) is a complete Kinase assay solution

ADP-Glo™ Kinase Assay 0-1mM ATP

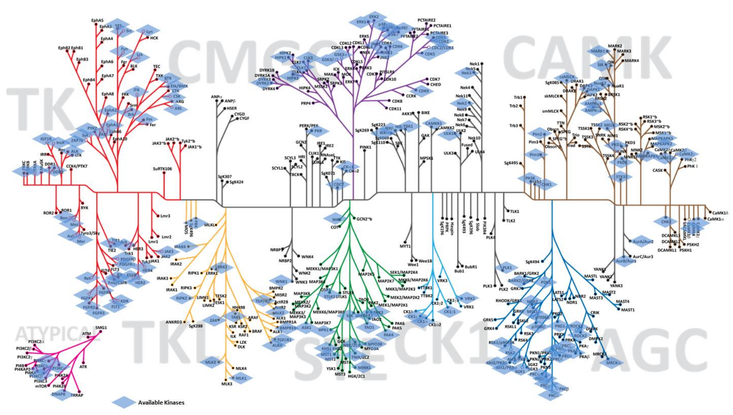
0.5ml	10mM UltraPure ATP
0.5ml	10mM ADP
5ml	ADP-Glo Reagent
10ml	Kinase Detection Buffer
1cake	Kinase Detection Substrate

+

Akt1 Kinase Enzyme System (Example)

0.1ml	AKT1 Kinase (10µg)
1ml	AKT (SGK) substrate (1mg)
1.5ml	Kinase Assay buffer
25µl	100mM DTT
25µl	2.5M MnCl2*
500µl	Kinase Activator**

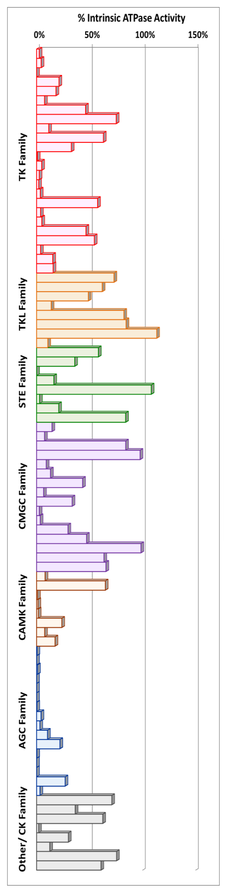
*For Tyrosine Kinases
**Lipids for PKC Kinases

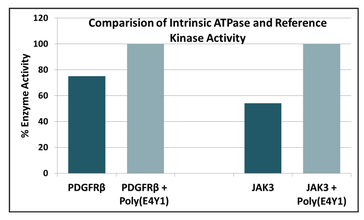


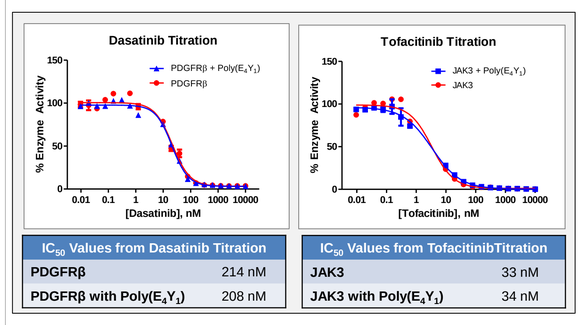
Broad Human Kinome coverage with >170 KES

4. Optimal performance ensured by reagent purity.

Genuine Kinase activities validated through specific inhibitions and intrinsic ATPase activity evaluation







IC_{50} Values from Dasatinib Titration	
PDGFRβ	214 nM
PDGFRβ with Poly(E_4Y_1)	208 nM

IC_{50} Values from TofacitinibTitration	
JAK3	33 nM
JAK3 with Poly(E_4Y_1)	34 nM

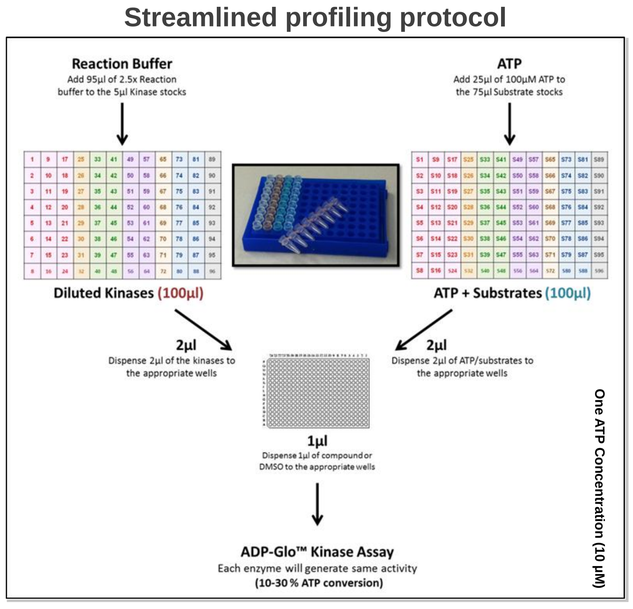
- **Kinases have different levels of intrinsic ATPase activity in vitro.**
- **The Kinases and their ATPase activities are completely inhibited by pan or selective inhibitors consistent with high purity.**
- **Specific inhibitors have the same potency (IC_{50}) towards the kinase or its intrinsic ATPase activity confirming the accuracy of inhibitor profiles.**

5. Kinase strips make profiling with ADP-Glo™ platform simple.

Important kinase targets organized in multi-well strip panels

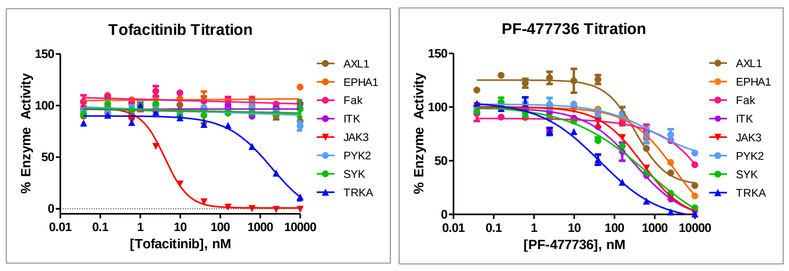
1	2	3	4	5	6	7	8	9	10	11	12	General Panel		
EGFR	ABL1	AXL1	CDK1/CyclinA2	ERK2	AKT1	PKCa	CHK1	Aurora A	AMPK A1/B1/G1	ALK2	ASK1	FGFR1	CDK2/CyclinA2	AKT1
HER2	BRK	EPHA1	CDK2/CyclinA2	GSK3b	p70S6K	PKCb II	CHK2	Aurora B	AMPK A1/B1/G2	ALK4	HPK1	JAK3	GSK3b	PKCa
HER4	BTK	FAK	CDK3/CyclinE1	JNK1	PDK1	PKCd	MAPKAPK2	CK2a1	AMPK A2/B1/G1	LRRK2	MINK1	LCK	p38a	ROCK1
IGF1R	CSK	ITK	CDK5/p25	JNK3	PKA	PKCe	MARK1	DNA-PK	CAMK2a	MLK2	MST1	SYK	AMPK A1/B1/G2	Aurora A
InsR	FYN A	JAK3	CDK5/p35	p38a	PKC	PKCg	MELK	CK1α1	CAMK2g	RIPK2	NIK	MINK1	CAMK4	CK2a1
KDR	LCK	PYK2	CDK6/Cyclin D3	p38b	PKG	PKCi	PASK	CK1epsilon	CAMK4	TGFbR1	PAK1/cocα	PAK1/cocα	CHK1	IKKa
PDGFRa	LYN B	SYK	CDK9/Cyclin K	p38d	ROCK1	PKCtheta	PIM1	CK1g1	DAPK1	IRAK4	SLK	IRAK4	DAPK1	CK1α1
PDGFRb	SRC	TRKA	CLK3	p38g	RSK2	PKCz	PKCmu (PKD1)	VRK2	STK33	TAK1-TAB1	TNIK	TAK1-TAB1	MAPKAPK2	CK1g1

Streamlined profiling protocol



6. Simple protocol for flexible and targeted inhibitor profiling.

Dose response for 2 compounds against 1 Kinase Profiling Strip



One Kinase Profiling Strip tested with 8 compounds at a single dose

	Kinase	Gefitinib	Dasatinib	Tofacitinib	SB203580	Roscovitine	PF-477736	Tozasertib	Enzastaurin
ST-3 TK family	AXL1	108	92	105	94	102	54	107	101
	EPHA1	84	1	102	79	97	63	77	100
	Fak	95	84	118	106	109	86	91	115
	ITK	102	101	99	94	100	33	24	99
	JAK3	99	113	0	87	94	36	88	103
	PYK2	133	134	122	106	134	120	80	116
	SYK	104	72	84	87	94	34	77	103
	TRKa	95	82	60	89	87	9	1	89

Inhibitor Concentration = 1 µM

- **Enabling flexible Kinase Profiling with the Strip Systems.**
- **Dose response or single dose profiling against 8 kinases at once.**

7. Creating selectivity profiles of inhibitors with ADP-Glo™ platform.

Inhibitor profiling performed on a large scale using a single dose compound profiling protocol

ADP-Glo™ profiling systems | Published radiometric data[1]

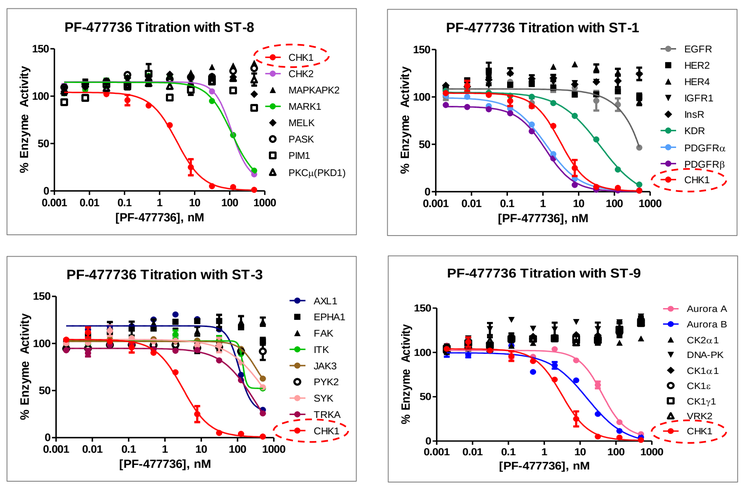
Strip #	Kinase	SB203580	Enzastaurin	PF-477736	Gefitinib	Dasatinib	Tofacitinib	Roscovitine	Tozasertib	Dasatinib *	Tofacitinib *	Roscovitine *	Tozasertib *	Kinase
ST-1 TK Family	EGFR	99	104	43	2	64	102	94	103	21	96	97	78	EGFR
	HER2	87	106	102	18	106	89	97	101	80	109	103	102	HER2
	HER4	96	104	64	28	13	103	101	98	4	100	98	93	HER4
	IGF1R	102	105	98	103	100	102	96	97	97	101	98	44	IGF1R
	InsR	98	100	95	97	94	97	101	88	97	97	94	52	InsR
	KDR	84	93	4	86	83	83	91	34	103	106	97	85	KDR
	PDGFRa	94	84	1	62	2	90	93	36	2	104	98	71	PDGFRa
	PDGFRb	85	88	0	85	1	77	92	40	2	95	91	72	PDGFRb
ST-2 TK Family	ABL1	98	102	27	97	0	106	107	20	3	101	102	14	ABL1
	BRK	54	105	36	73	4	115	105	92	5	101	103	95	BRK
	BTK	99	99	33	83	5	101	111	46	2	99	83	99	BTK
	CSK	95	105	41	97	3	99	102	81	7	101	89	78	CSK
	FYN A	90	93	8	74	0	67	97	30	2	94	106	72	FYN A
	LCK	90	88	22	74	2	89	92	27	0	80	81	12	LCK
	LYN B	95	91	10	75	0	97	96	78	2	99	105	63	LYN B
	SRC	89	96	10	90	0	98	91	52	4	100	107	27	SRC
ST-3 TK Family	AXL1	94	101	54	108	92	105	102	107	61	98	100	44	AXL1
	EPHA1	79	100	63	84	1	102	97	77	3	98	97	78	EPHA1
	Fak	106	115	86	95	84	118	109	91	103	103	96	100	Fak
	ITK	94	99	33	102	101	99	100	24	102	99	105	99	ITK
	JAK3	87	103	36	99	113	0	94	88	93	2	97	92	JAK3
	PYK2	106	116	120	133	134	122	134	80	98	100	100	95	PYK2
	SYK	87	103	34	104	72	84	94	77	65	107	103	99	SYK
	TRKa	89	89	9	95	82	60	87	1	97	94	98	7	TRKa
ST-4 CMGC Family	CDK1/Cyclin A2	111	109	89	95	85	112	64	102	96	96	83	99	CDK1/Cyclin A2
	CDK2/Cyclin A2	99	101	64	111	99	77	24	96	92	86	24	88	CDK2/Cyclin A2
	CDK3/Cyclin E1	89	90	92	99	116	99	44	106	92	92	58	95	CDK3/Cyclin E1
	CDK5/p25	97	98	75	89	104	100	26	99	102	100	31	94	CDK5/p25
	CDK5/p35	101	105	75	107	99	96	25	100	100	99	31	100	CDK5/p35
	CDK6/Cyclin D3	104	96	79	106	111	93	84	102	103	111	96	121	CDK6/Cyclin D3
	CDK9/Cyclin K	110	72	83	100	93	104	41	110	84	100	79	93	CDK9/Cyclin K
	CLK3	90	98	87	106	92	98	98	92	95	106	105	110	CLK3
ST-5 CMGC Family	ERK2	97	105	20	103	99	109	99	102	93	89	88	91	ERK2
	GSK3b	55	-3	86	86	103	105	96	102	97	100	96	104	GSK3b
	JNK1	84	107	95	89	99	104	107	97	106	108	98	95	JNK1
	JNK3	30	106	86	81	95	93	106	88	100	100	101	107	JNK3
	p38a	3	102	99	64	24	105	101	103	37	93	96	97	p38a
	p38b	10	103	94	91	54	100	101	105	77	103	101	93	p38b
	p38d	105	105	95	105	101	95	107	109	99	94	97	90	p38d
	p38g	94	104	98	112	101	103	104	107	100	97	95	96	p38g
ST-7 AGC Family	PKCa	96	5	77	88	107	106	103	102	103	79	87	82	PKCa
	PKCb II	105	4	75	91	101	100	105	95	89	88	96	90	PKCb II
	PKCd	97	11	77	71	106	90	97	92	100	75	102	98	PKCd
	PKCe	94	9	66	99	97	97	106	100	102	99	98	98	PKCe
	PKCg	100	14	37	89	96	78	101	89	86	91	96	94	PKCg
	PKCi	93	97	84	101	97	99	98	99	101	103	92	99	PKCi
	PKCtheta	102	-3	66	97	95	87	100	102	97	89	90	86	PKCtheta
	PKCz	101	92	85	108	98	95	95	116	101	92	100	76	PKCz
ST-8 CAMK Family	CHK1	97	97	0	95	96	105	100	101	96	94	94	77	CHK1
	CHK2	100	106	8	80	98	107	109	90	103	104	97	100	CHK2
	MAPKAPK2	103	106	100	91	98	101	106	98	100	97	101	103	MAPKAPK2
	MARK1	93	96	6	97	97	78	103	97	107	119	115	114	MARK1
	MELK	98	109	43	101	106	86	94	14	81	79	85	53	MELK
	PASK	98	99	90	101	111	93	96	92	110	105	93	99	PASK
	PIM1	108	70	65	96	112	99	96	104	102	102	105	99	PIM1
	PKCmu (PKD1)	97	98	76	82	102	98	97	97	99	97	99	102	PKCmu (PKD1)
ST-9 Other/CK Family	Aurora A	99	108	4	102	95	80	105	4	93	84	89	5	Aurora A
	Aurora B	93	111	3	95	94	94	105	2	93	99	95	5	Aurora B
	CK2a1	94	115	86	103	108	105	105	99	105	104	106	114	CK2a1
	DNA-PK	102	91	85	106	104	99	113	115	N/A	N/A	N/A	N/A	DNA-PK
	CK1a1	86	103	93	104	98	102	95	107	98	100	91	97	CK1a1
	CK1epsilon	58	95	96	100	105	96	86	107	90	101	81	99	CK1epsilon
	CK1g1	92	99	97	102	105	94	88	104	97	101	94	99	CK1g1
	VRK2	91	103	96	103	107	99	94	109	98	100	107	107	VRK2

Inhibitor Concentration = 1µM | * Inhibitor Concentration = 0.5µM

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

Data generated with ADP-Glo™ platform consistent with published potencies of radioactivity-based Kinome profiling[1].

Confirming promiscuity of PF-477736 compound using the dose response profiling protocol



IC_{50} Values from PF-477736 Titration			
PDGFRα	1.2 nM	Aurora A	41.4 nM
PDGFRβ	1.2 nM	MARK1	113.8 nM
CHK1	3.1 nM	CHK2	133.8 nM
Aurora B	35.8 nM	TRKa	299.4 nM
KDR	39.7 nM	JAK3	316.6 nM

- **Corresponding Dose-Response curves confirm PF-477736 promiscuity.**
- **IC_{50} values demonstrate potency of PF-477736 towards different targets other than CHK1.**
- **Promiscuity of specific inhibitors is identified and confirmed with the profiling strips protocols**

8. Conclusion

Kinase Profiling Strip Systems have the following advantages:

- ✓ ***Fast and simple reaction assembly*: Two quick dilutions provide working stocks of kinase and substrate/co-factor solutions sufficient for 50 kinase reactions.**
- ✓ ***One-time use design*: Eliminating multiple freeze/thaw cycles ensures optimal kinase activity for each experiment.**
- ✓ ***Optimized kinase activity for inhibitor profiling*: All kinases have been optimized to provide 10-30% ADP production when assayed at 10µM ATP.**
- ✓ ***Formatted strips provide access to eight kinases at a time*: Kinases from singular kinase families are grouped together for more relevant selectivity profiles.**
- ✓ ***Flexible kinase inhibitor profiling*: Each strip has enough material to profile 8 compounds at a single dose or create a dose response for 2 compounds against 8 kinases at once.**

1- Anastassiadis, T. et al;. Nat. Biotechnol. 2011, 29, 1039