

# ADP-Glo™ Kinase Profiling Systems for Targeted and Flexible Kinase Inhibitor Profiling



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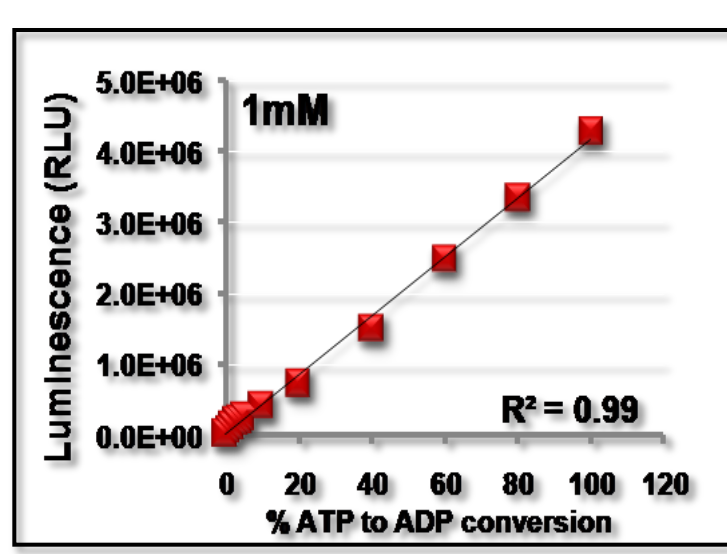
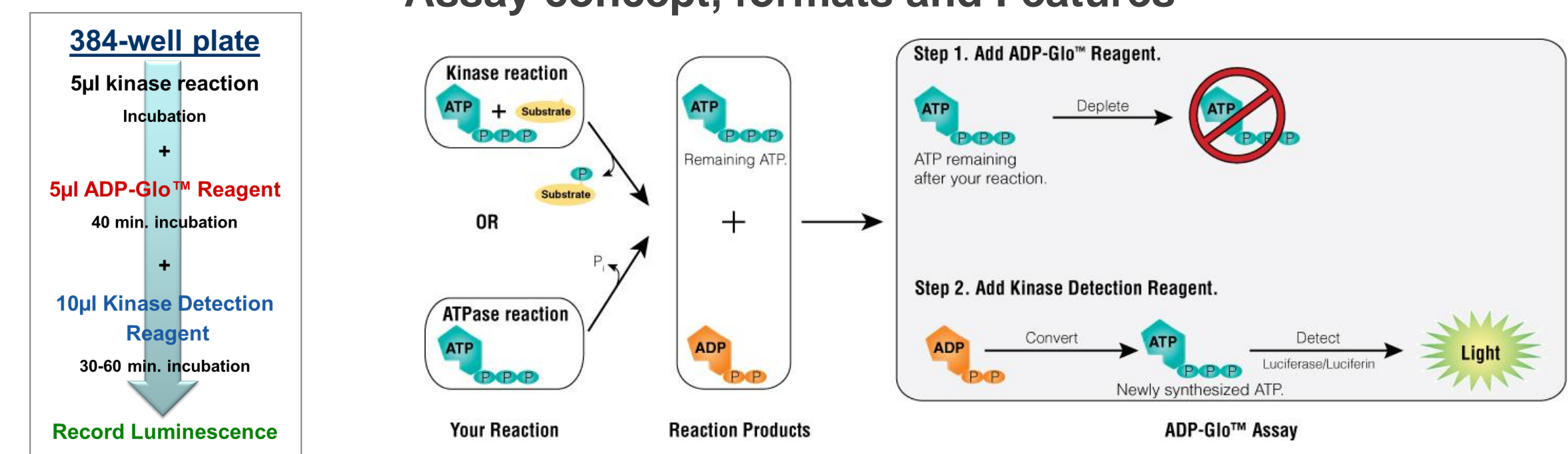
Abstract #176

## 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

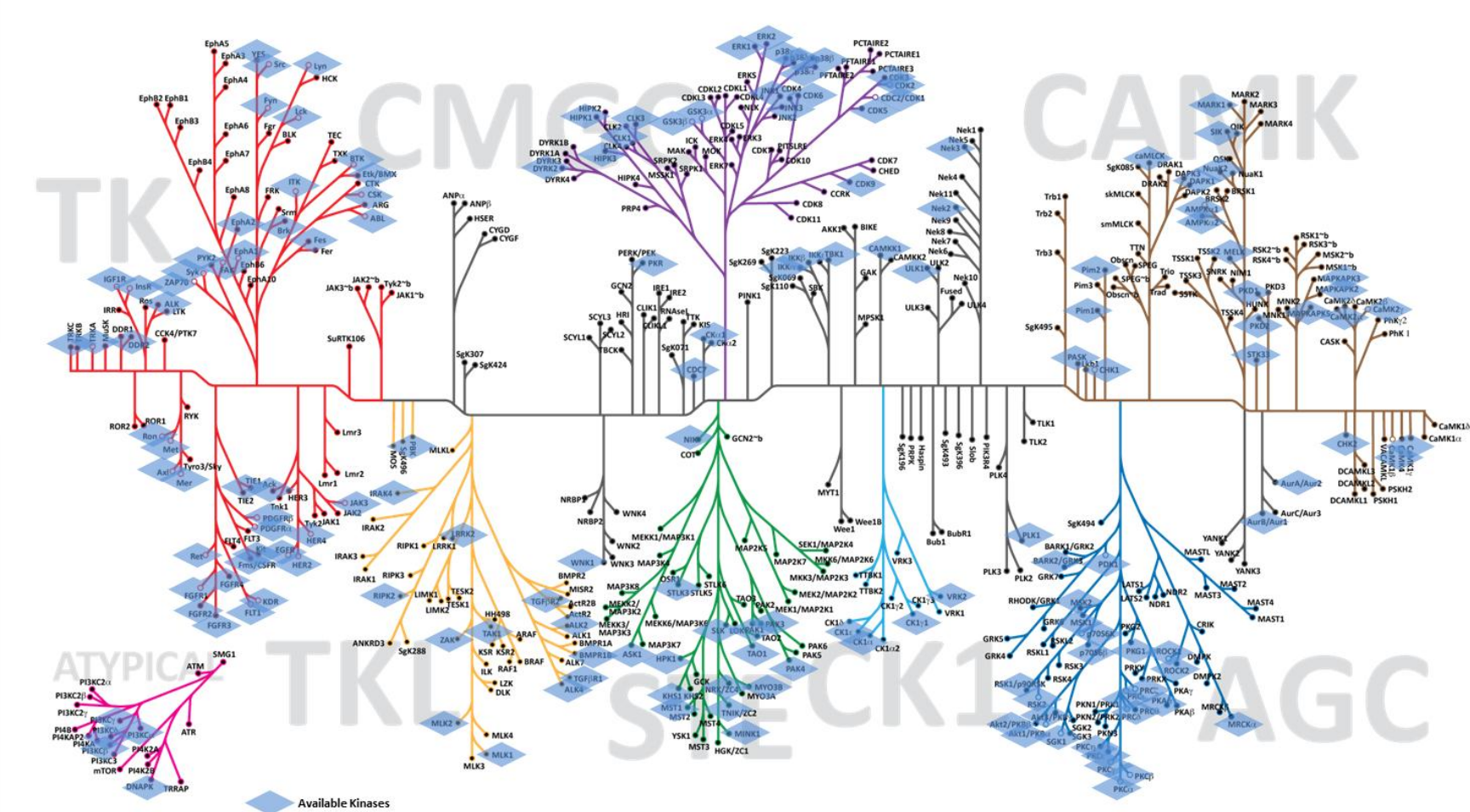


- **Universal:** Any kinase-substrate combination.
- **Wide dynamic range:** High sensitivity at low % ATP to ADP conversion allows use of lower amount of enzyme.
- **Broad range of [ATP]:** (µM to mM) allows distinction between ATP competitive and non competitive inhibitors.

## 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 Reagent
- 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\* For Tyrosine Kinases
  - 500µl Kinase Activator\*\* \*\*Lipids for PKC Kinases



Broad Human Kinome coverage with >170 KES

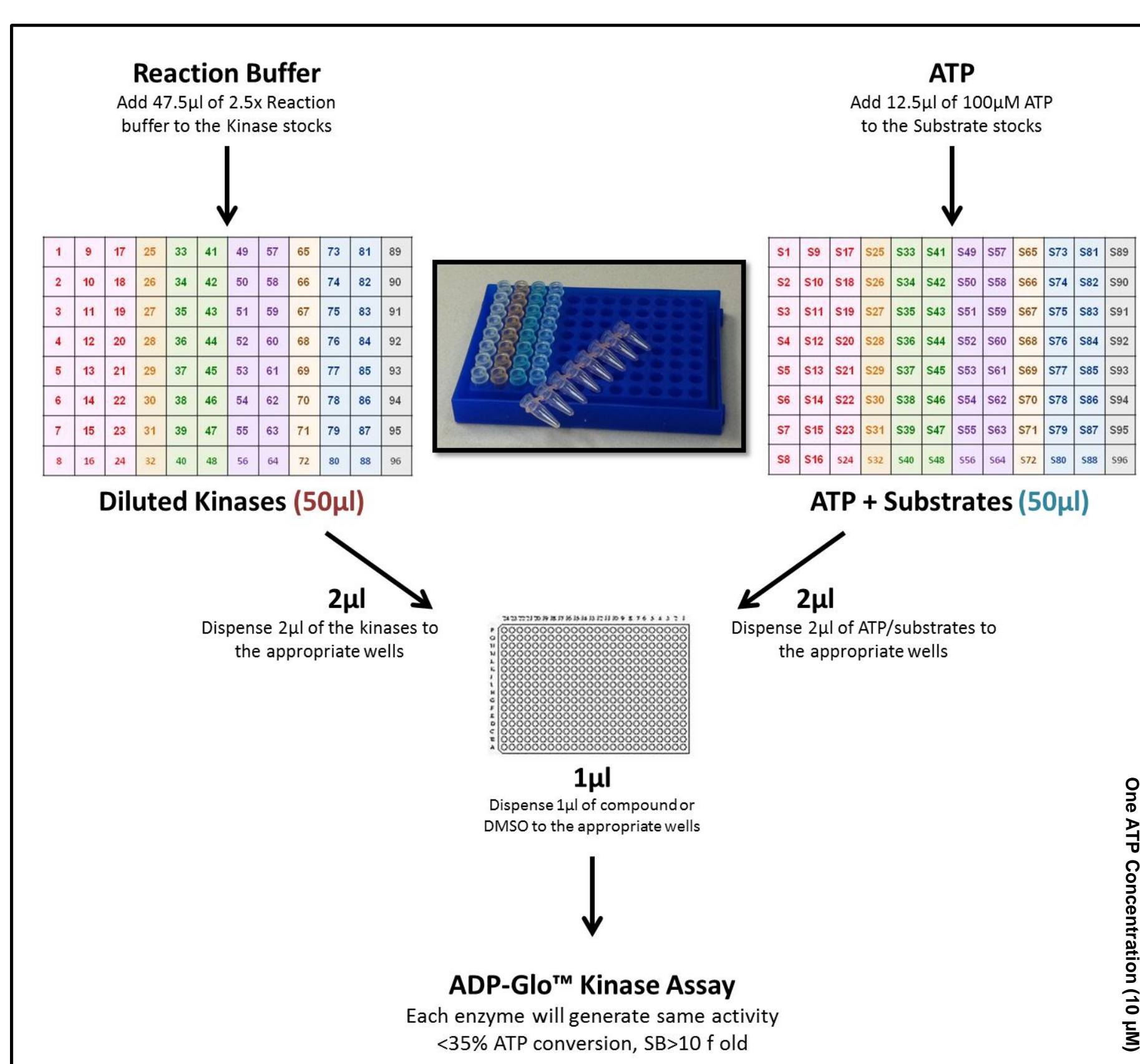
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## 4. Simple protocol for flexible and targeted inhibitor profiling

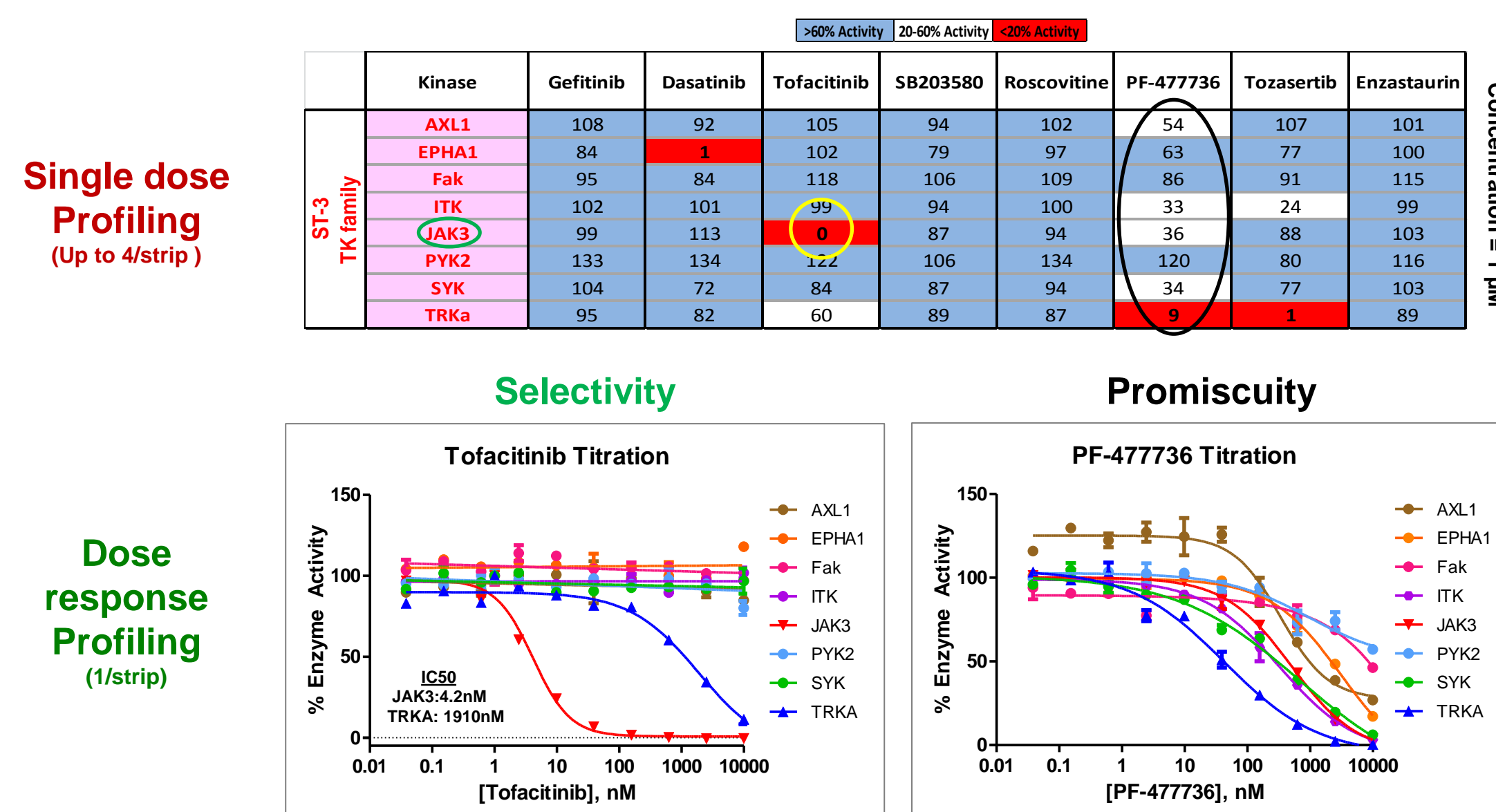
Important kinase targets organized in multi-well strip panels

Family based strips												General panel		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
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	PKD1	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
Inr	FYN A	JAK3	CDK5/p25	p38a	PKC	PKCg	MELK	CK1a1	CAMK2g	RIPK2	NIK	MINK1	CAMK4	CK2a1
KDR	LCK	PKY2	CDK6/Cyclin D3	p38b	PKG	PKCI	PASK	CK1epsilon	CAMK4	TGFbR1	PAK1/coxa	PAK1/coxa	CHK1	IKKa
PDGFRa	LYN B	SYK	CDK9/Cyclin K	p38d	ROCK1	PKCtheta	PIM1	CK1g1	DAPK1	IRAK4	SLK	IRAK4	DAPK1	CK1a1
PDGFRb	SRC	TRKA	CLK3	p38g	RSK2	PKCz	PKCmu (PKD1)	VRK2	STK33	TAK1-TAB1	TNIK	TAK1-TAB1	MAPKAPK2	CK1g1

### Streamlined profiling protocol



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



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

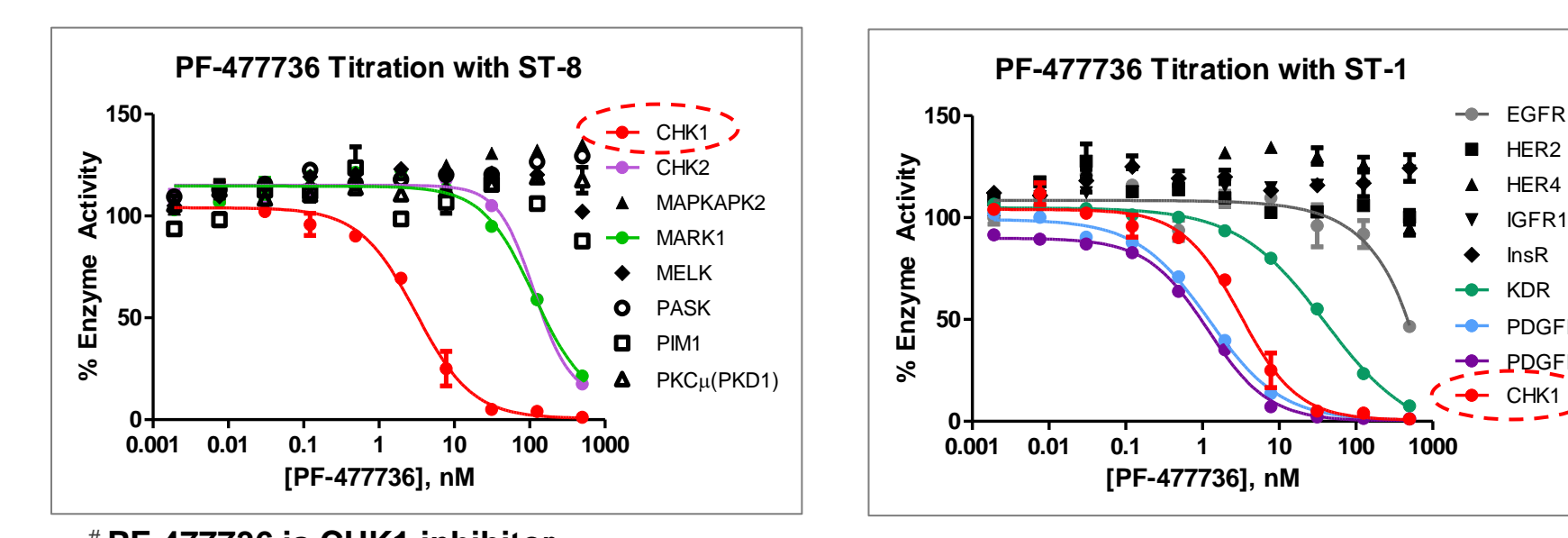
## 6. Creating selectivity profiles of inhibitors with ADP-Glo™ Kinase Profiling platform

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

Strip #	Kinase	ADP-Glo™ profiling systems				Published radiometric data <sup>1</sup>			
		SB203580	Dasatinib	PF-47736	Genistein	Dasatinib	Tofacitinib	Roscovitine	Tozasertib
ST-3 TK family	EGFR	99	104	41	7	94	102	94	103
	HER2	87	104	102	88	100	89	97	103
	HER4	96	104	64	102	95	100	100	98
	IGF1R	100	100	98	103	100	102	96	97
	Inr	100	95	100	99	99	97	101	96
	KDR	84	93	86	83	83	91	84	83
	PDGFRa	94	94	90	90	94	90	90	90
	PDGFRb	85	88	88	85	77	83	85	83
	SYK	95	95	95	95	95	95	95	95
	SRC	89	96	74	90	74	98	111	112

Data generated with ADP-Glo™ platform consistent with published potencies of radioactivity-based Kinome profiling<sup>1</sup>.

### Confirming promiscuity of PF-47736# compound using the dose response profiling protocol



## 7. 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 25 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 <35% ADP production with >10 fold S/B.
- ✓ **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 4 compounds at a single dose or create a dose response for 1 compound against 8 kinases at once.

<sup>1</sup> Anastassiadi, T. et al.; Nat. Biotechnol. 2011, 29, 1039