Flexible and targeted inhibitor profiling using the luminescent ADP-Glo™ kinase assay platform

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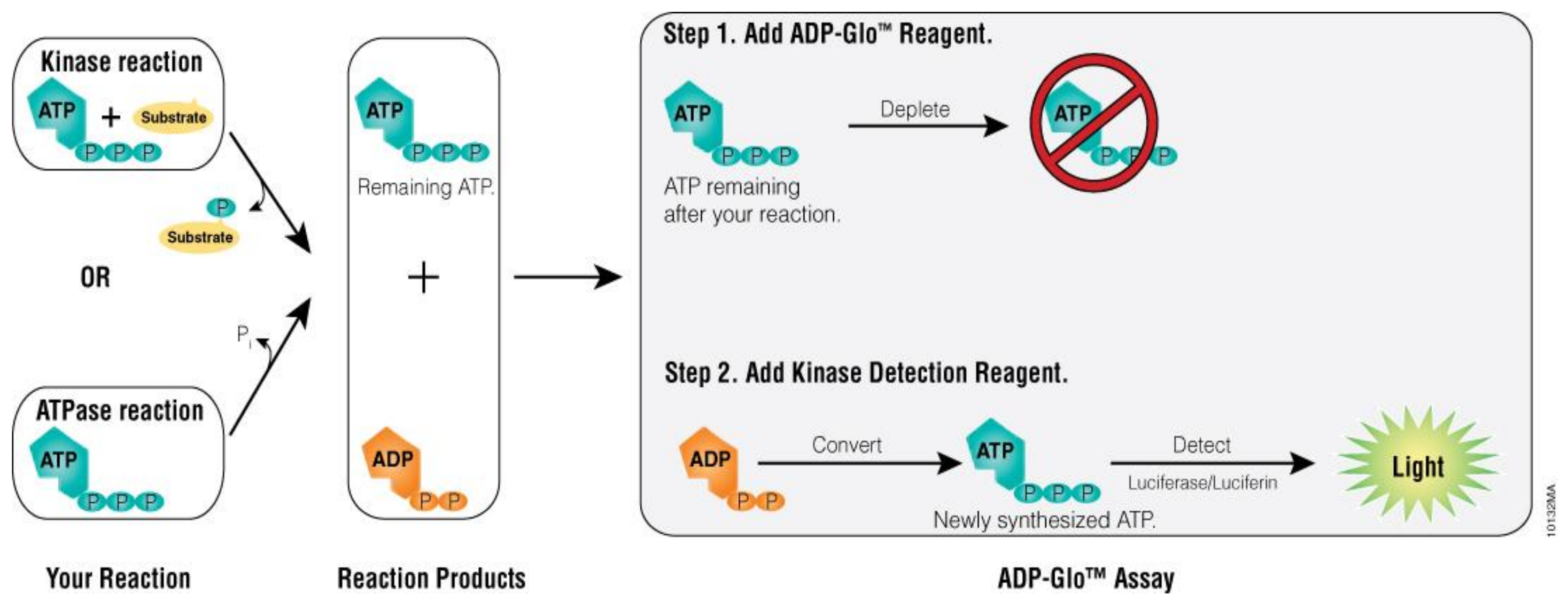
1. Abstract

Profiling kinase inhibitors is a critical step during drug development. Knowing the inhibitory profile of a compound across a broad collection of kinases can be important for better understanding its biological activity, obviating any off-target activities, and in some cases identifying new targets may lead to novel therapeutic applications. Unlike an HTS phase, where a target-specific kinase assay is used to identify hit compounds, during profiling a robust universal assay is needed to assess the selectivity and potency of the inhibitor on multiple kinases from different classes. Often, these kinases use substrates with different chemical structures and target-specific screening assays are not suitable or can be cost prohibitive. We previously introduced the luminescent ADP-Glo™ kinase assay that measures kinase activity by quantifying the amount of ADP produced during the enzymatic reaction. This technology addressed all the needs of kinase screening, mode of action (MOA) studies and profiling using one assay format during the drug discovery process. Here, we show that we can achieve the sensitivity and robustness required for profiling the different kinase families covering the human kinome with one platform. The ADP-Glo™ assay was previously validated with more than hundred kinases and is now optimized for use with a large panel of complete Kinase Enzyme Systems (KES) that span different families of the human kinome. We also demonstrate the profiling of different kinase inhibitors using newly designed kinase strips. Each multi-well strip contains a group of kinases from the same family. The kinase stock volumes were standardized in a way that, when all the kinases were diluted into the kinase reaction, the kinases generated 5–10% ATP to ADP conversion. The substrate stocks were standardized in similar fashion and are located in a second strip at corresponding positions. We show that using the new kinase profiling strips and an optimized protocol, we could easily generate selectivity profiles using small or large kinase panels, as well as detecting compound promiscuity towards members of a single kinase subfamily or different subfamilies of the kinome. The fact that ADP-Glo™ platform offers so many positive attributes makes it an ideal assay not only for primary and secondary screening but also for profiling compounds in a cost-effective manner using one single platform.

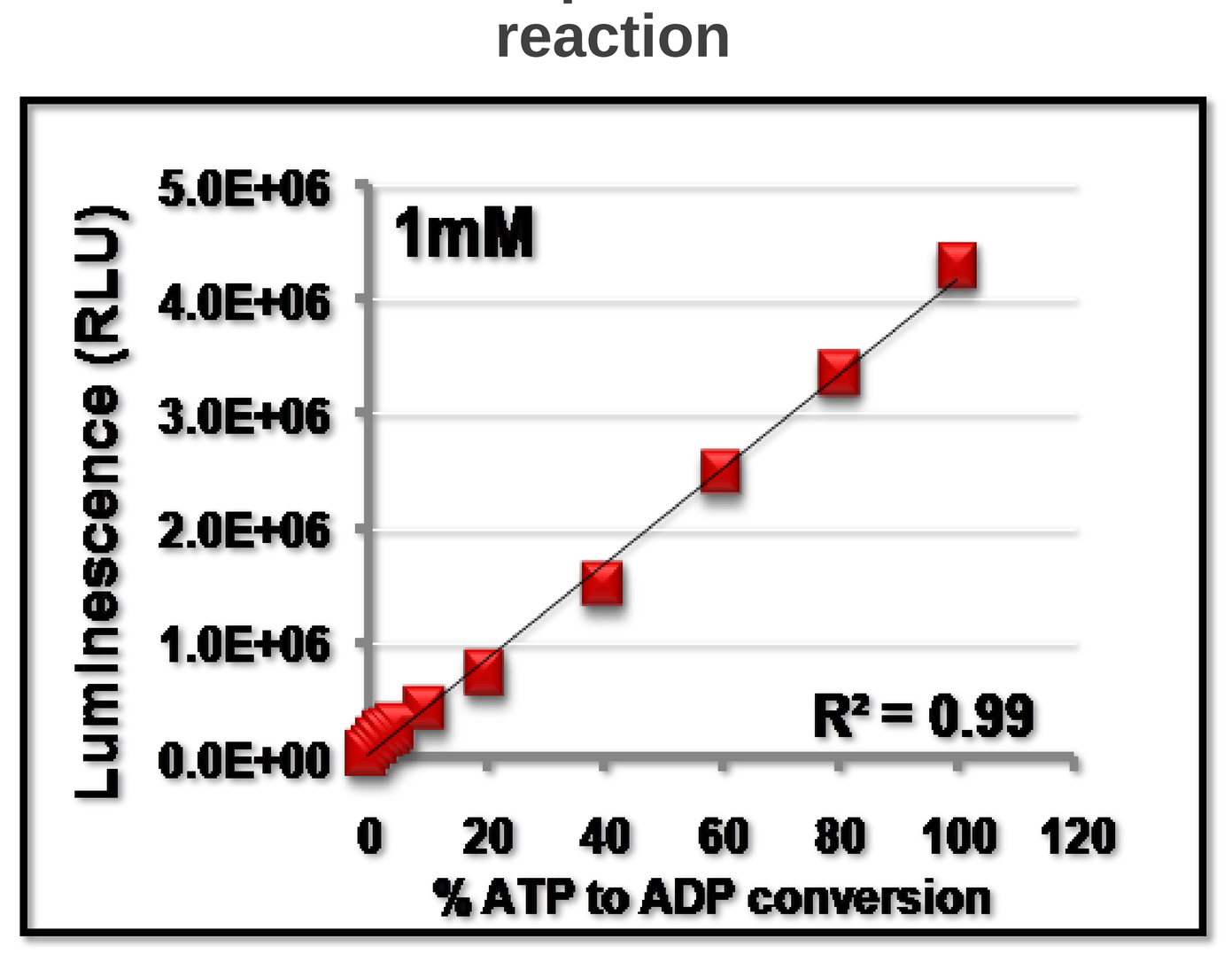
2. ADP-Glo™ is a positive detection assay for product formation



- Step 1: Depletion of unconsumed ATP after the kinase reaction.
- Step 2: ADP is converted into ATP that is detected via a luciferase/luciferin reaction.
- Luminescent signal is proportional to ADP produced and the kinase activity.

3. ADP-Glo™ can be used with a broad range of ATP concentrations

ADP detection up to 1mM ATP in the reaction



More sensitivity with Promega new ultrapure ATP compared to other ATP sources

	ATP-ADP ranges								
	1mM			100µM			10µM		
% ADP in ATP + ADP mixture	20%	10%	5%	20%	10%	5%	20%	10%	5%
Promega	45	23	13	54	32	18	37	20	10
Competitor S	26	13	8	35	21	11	31	16	8
Competitor T	13	7	4	15	10	5	16	9	5
Competitor G	12	6	4	16	10	6	18	10	6

- Sensitivity: ADP-Glo™ generates wide dynamic range with Z'>0.7
- Linearity: ADP-Glo™ Kinase assay detects up to 1mM ADP in a linear fashion
- ADP-Glo™ sensitivity is highly improved with the new Ultra Pure ATP

4. ADP-Glo™ is a universal assay validated with large kinase panel spanning the kinome

ADP-Glo™ Kinase Assay platform: Universal Kinase Assay & 150+ KES*

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ADP-Glo™ Kinase Assay

+

Promega Kinase Enzyme Systems

(KES Example)

SignalChem

Specialists in Signaling Proteins

0.1ml	Akt1 Kinase (10µg)
1ml	Akt (PKB) substrate (1mg)
1.5ml	5 x Reaction Buffer A

*Colored Kinase names: Available now
Non colored Kinase names: Available soon

PI3K class I enzymes:

p110α/p85α
p110α (E545K)/p85a
p110α (H1047R)/p85α
P110β/ p85α
P110γ/ p85α
P110δ/ p85α

+ PIP2:PS or PI:PS Lipid Kinase substrate 1mM

TK					TK mutants	
ABL1	FES	ITK	RET	FGFR2	EGFR L858R	KIT T670I
AXL	FGFR1	JAK3	RON	FGFR4	EGFR L861Q	PDGFRA D842V
BTK	FLT1	KDR	SRC	ACK	EGFR T790M	PDGFRA T674I
c-MER	FYN A	LCK	SYK	DDR2	EGFR T790M L8	RET Y791F
CSK	HER2	LYN B	TRKA	PYK2	ABL1 E255K	RET V804L
EGFR	HER4	MET	ZAP70	c-Kit	ABL1 G250E	MET M1250T
EPHA1	IGF1R	PDGFRa	FLT3	TRKB	ABL1 T315I	FGFR3 K650E
FAK	InsR	PDGFRb	FMS	BRK	ABL1 Y253F	FLT3 D835Y

TKL		CAMK		
IRAK4	ALK1	AMPK A1/B1/G1	PIM1	MYLK3/caMLCK
TGFβR2	MLK1	CAMK2g	CAMK1g	STK33
TGFbR1	RIPK2	CAMK4	MLCK/MYLK	CHK2
BRAF	BMPR1b (ALK6)	CHK1	CAMK2a	PKCmu (PKD1)
BRAF V600E	TAK1-TAB1	AMPK A2/B1/G1	MAPKAPK2	PASK
RAF1(EE)	ALK2	MAPKAPK5	MAPKAPK3	MELK
MLK2	ZAK	PIM2	DAPK1	MARK1
TOPK	LRRK2	PKD2	SIK	AMPK A1/B1/G2

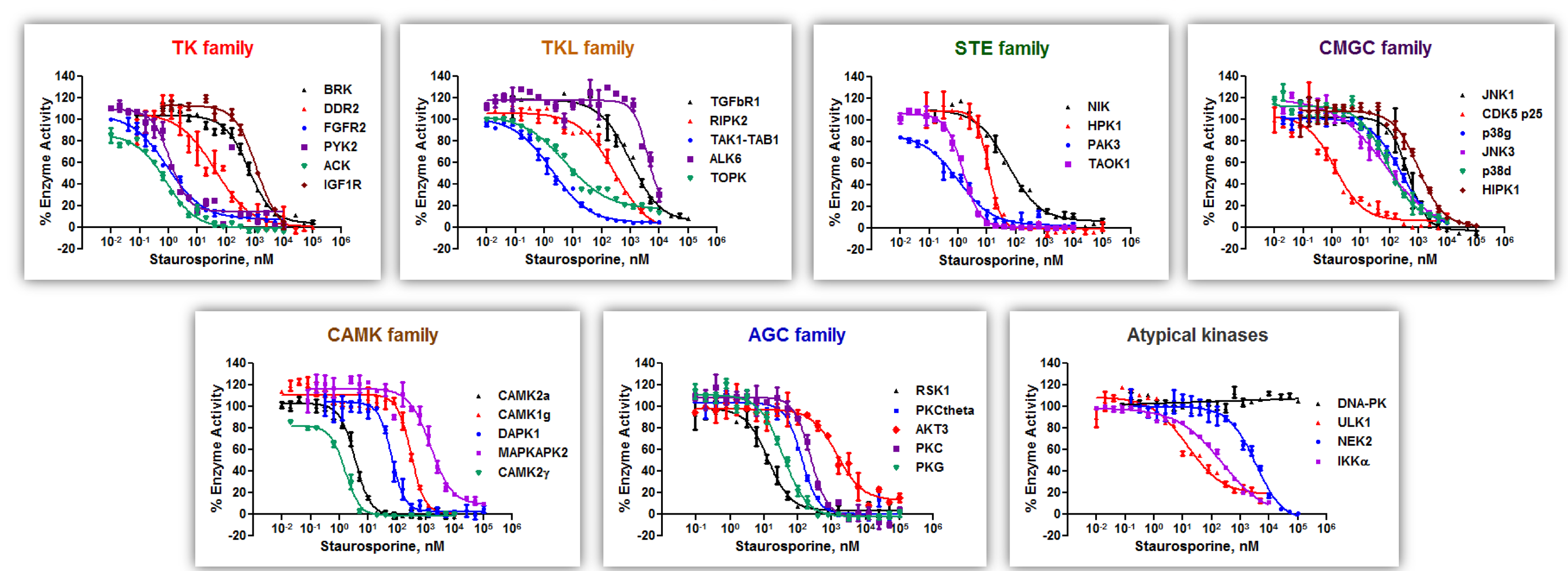
STE		CMGC		
ASK1	PAK1/CDC42	CDK1/CyclinA2	JNK3	CDK7
MINK1	MEKK1	CDK2/CyclinA2	p38a	CDK4/Cyclin D3
PAK4	MEKK2	CDK5/p25	p38g	CDK6/Cyclin D3
MEK1	MYO3b	CDK5/p35	CDK9/Cyclin K	CDK3/CyclinE1
PAK3	STK39/STLK3	ERK1	JNK1	CLK1
MST1	KHS1	ERK2	CDK2/Cyclin E1	CLK3
SLK	NIK	GSK3a	p38b	HIPK1
HPK1	TAOK1	GSK3b	p38d	HIPK3

AGC			OTHER/CK1	
AKT1	PKCg	RSK1	Aurora A	IKKa
AKT2	PKCi	PKCtheta	Aurora B	CK2a1
GRK5	PKCz	ROCK2	NEK2	CAMKK1
p70S6K	ROCK1	p70S6Kb	PLK1	CK1α1
PDK1	SGK1	GRK2	TBK1	VRK2
PKCa	RSK2	PKG	ULK1	CK1g2
PKCb II	AKT3	PKA	NEK6	CK1epsilon
PKCd	PKCe	PKC	DNA-PK	CK2

Larger kinase panel for easy profiling and selectivity assays

5. Profiling kinase inhibitors with ADP-Glo™ kinase assay

Staurosporine selectivity profiles of small kinase family panels

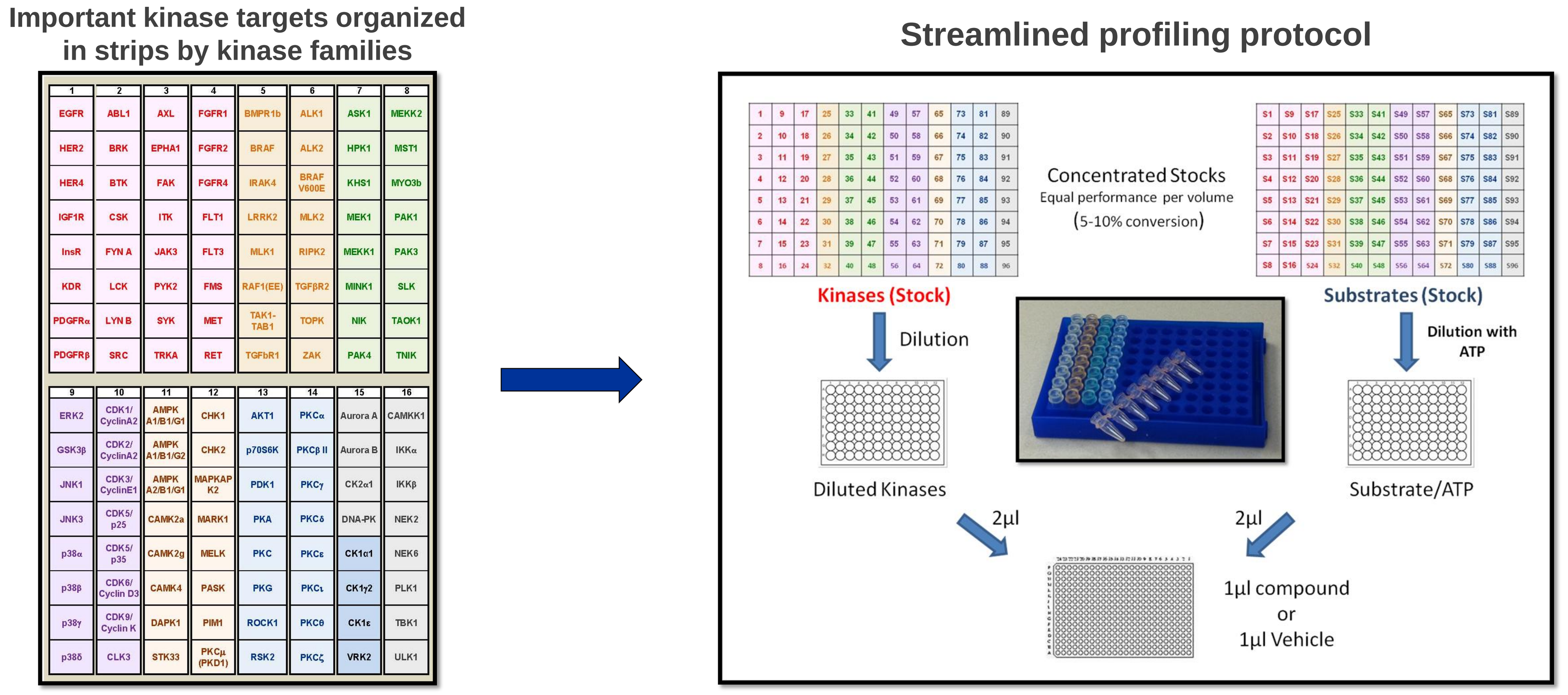


"One Assay for all", ADP-Glo™ is ideal for profiling inhibitors against large or small kinase panels

6. Kinase strips for flexible and targeted inhibitor profiling

Important kinase targets organized in strips by kinase families

Streamlined profiling protocol



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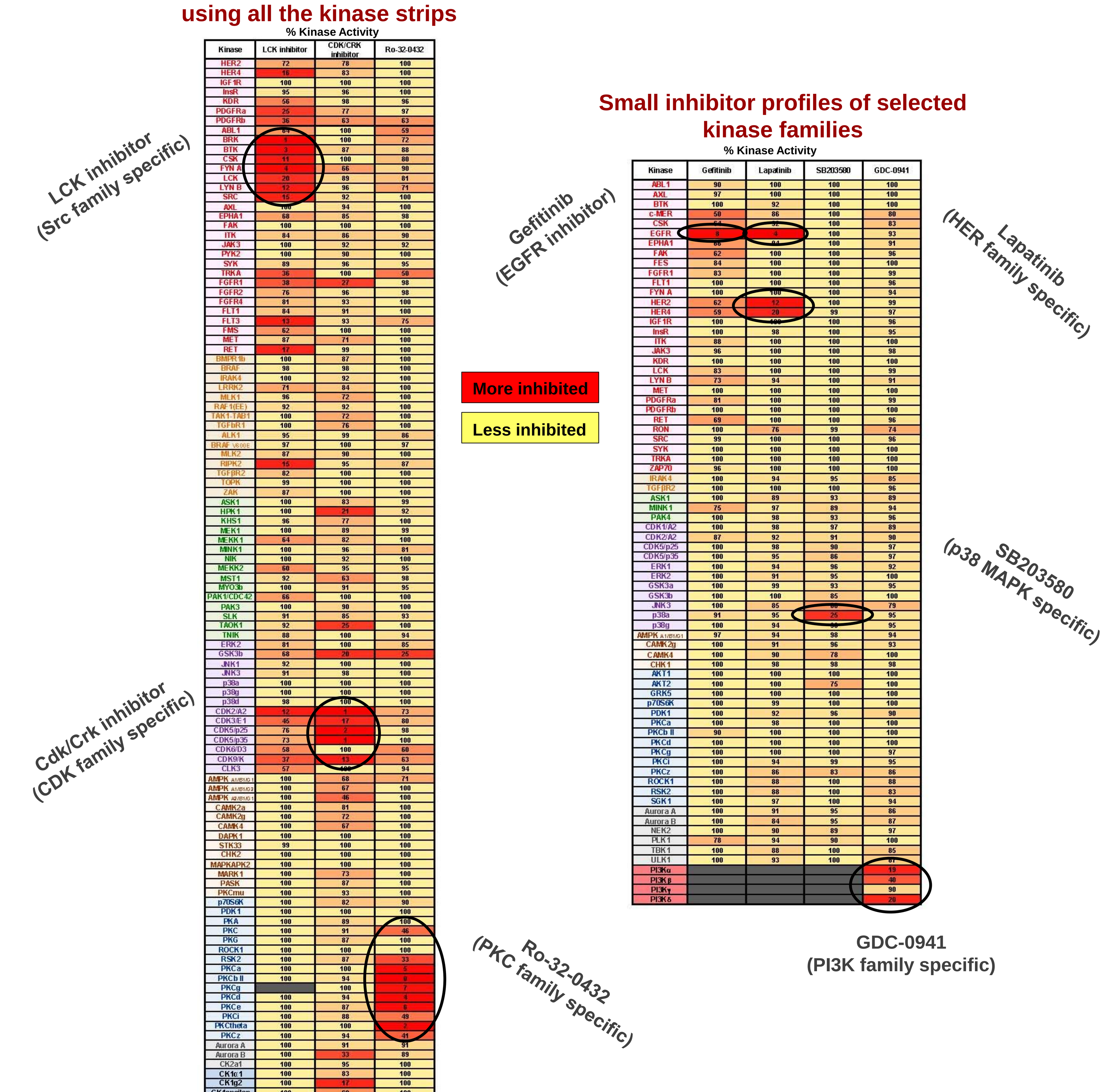
7. Determination of inhibitor profiles of different kinase families with ADP-Glo™ platform



ADP-Glo™ Kinase assay + KES + kinase strips for convenient and meaningful selectivity profiles creation

8. General features of ADP-Glo™ platform

- **High sensitivity assay**: less than 1% ATP conversion detected with more than 2.5 signal to background ratio with new Ultra Pure ATP
- **High dynamic range:** High signal to background at low % ATP to ADP conversion allows use of lower amount of enzyme during HTS or profiling
- **Homogenous, non radioactive and antibody Free**
- **Robust assay (Z' higher than 0.7)**
- **Universal assay:** Any ADP-producing enzyme (Kinase or ATPase). Ideal for inhibitor profiling with easy protocol.
- **Larger kinase panel:** Complete Kinase Enzyme Systems for easy profiling and selectivity assays
- **Convenient profiling concept:** Strips can be used in a mix and match fashion to profile representatives of a single kinase family or multiple families with the same assay platform

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