Streamlining Kinase Profiling Workflow by Integrating Bioluminescent Kinase Strips with Multimode Detection System

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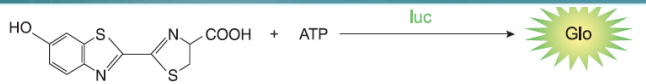
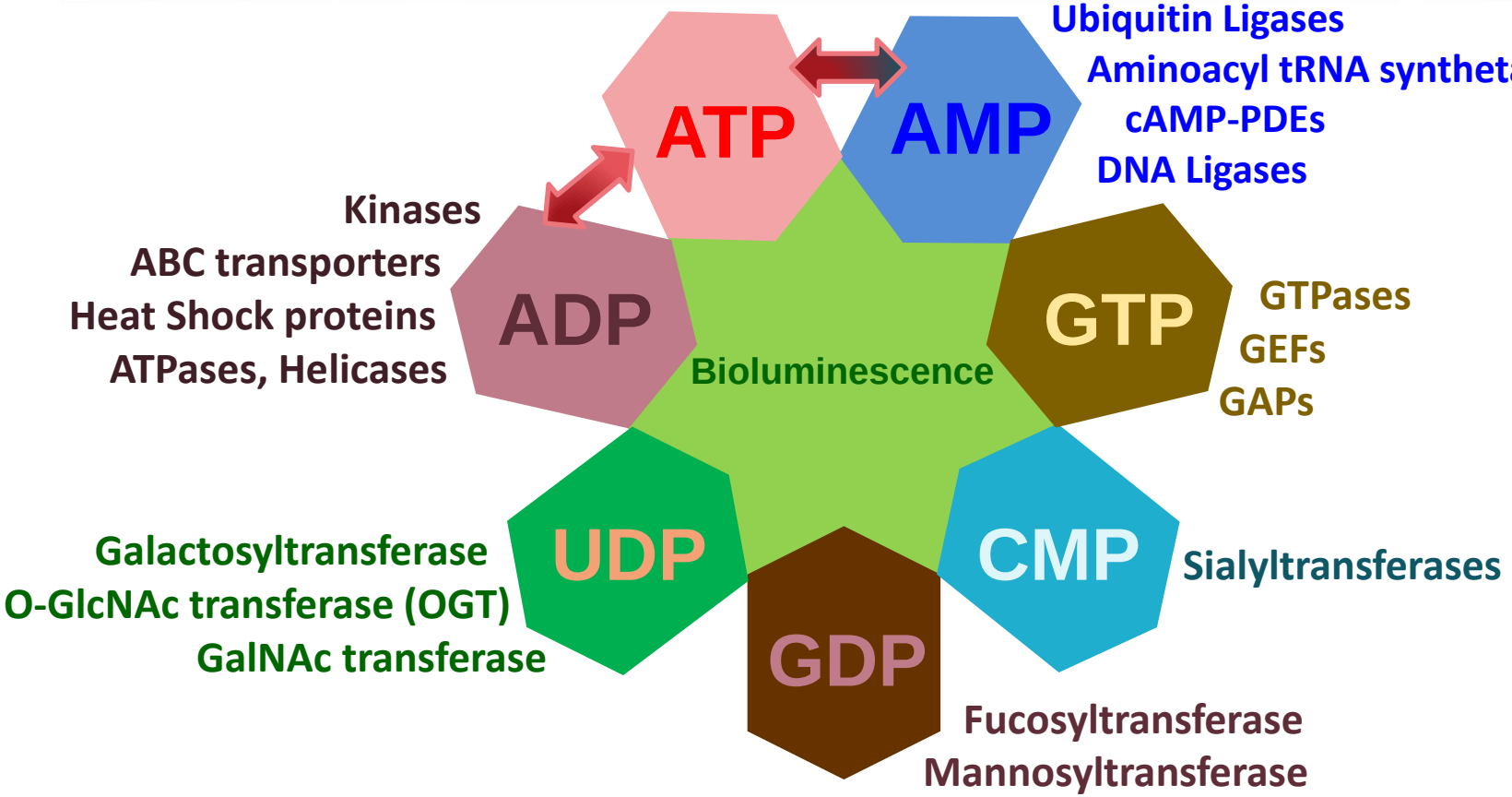
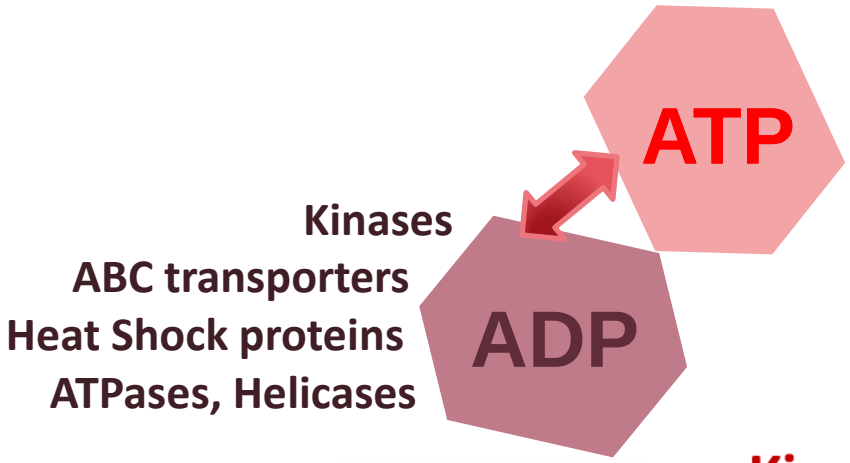


Nucleotides enzyme targets



Bioluminescent Nucleotide detection technologies → **Powerful research toolbox for various enzyme characterizations**

Nucleotides enzyme targets: ADP



Kinases play a critical role in Human biology

Kinases are directly involved in many diseases

Kinases became the largest target in the drug discovery market

Importance of Kinases in Drug Discovery

The Facts:

US Marketed Small Molecule Kinase Inhibitors

Table 1. Targeted Therapeutics in Cancer.*

Gene	Genetic Alteration	Tumor Type	Therapeutic Agent
Receptor tyrosine kinase			
EGFR	Mutation, amplification	Lung cancer, glioblastoma	Gefitinib, erlotinib
ERBB2	Amplification	Breast cancer	Lapatinib
FGFR1	Translocation	Chronic myeloid leukemia	PKC412, BIBF-1120
FGFR2	Amplification, mutation	Gastric, breast, endometrial cancer	PKC412, BIBF-1120
FGFR3	Translocation, mutation	Multiple myeloma	PKC412, BIBF-1120
PDGFRA	Mutation	Glioblastoma, gastrointestinal stromal tumor	Sunitinib, sorafenib, imatinib
PDGFRB	Translocation	Chronic myelomonocytic leukemia	Sunitinib, sorafenib, imatinib
ALK	Mutation or amplification	Lung cancer, neuroblastoma, anaplastic large-cell lymphoma	Crizotinib
c-MET	Amplification	Gefitinib-resistant non–small-cell lung cancer, gastric cancer	Crizotinib, XL184, SU11274
IGF1R	Activation by insulin-like growth factor II ligand	Colorectal, pancreatic cancer	CP-751,871, AMG479
c-KIT	Mutation	Gastrointestinal stromal tumor	Sunitinib, imatinib
FLT3	Internal tandem duplication	Acute myeloid leukemia	Lestaurtinib, XL999
RET	Mutation, translocation	Thyroid medullary carcinoma	XL184
Non–receptor tyrosine kinase			
ABL	Translocation (BCR-ABL)	Chronic myeloid leukemia	Imatinib
JAK2	Mutation (V617F), translocation	Chronic myeloid leukemia, myeloproliferative disorders	Lestaurtinib, INCB018424
SRC	Overexpression	Non–small-cell lung cancer; ovarian, breast cancer; sarcoma	KX2–391, dasatinib, AZD0530
Serine–threonine–lipid kinase			
BRAF	Mutation (V600E)	Melanoma; colon, thyroid cancer	SB-590885, PLX-4032, RAF265, XL281
Aurora A and B kinases	Overexpression	Breast, colon cancer; leukemia	MK-5108 (VX-689)
Polo-like kinases	Overexpression	Breast, lung, colon cancer; lymphoma	BI2536, GSK461364
MTOR	Increased activation	Renal-cell carcinoma	Temsirolimus (CCI-779), BEZ235
PI3K	PIK3CA mutations	Colorectal, breast, gastric cancer; glioblastoma	BEZ235

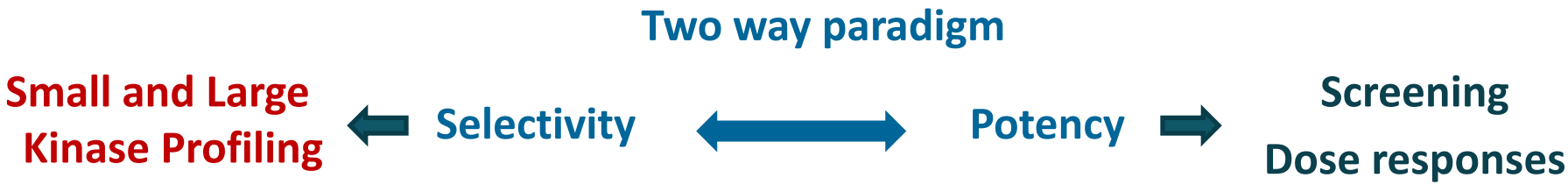
Diseases targeted:

- **Oncology,**
- **inflammation,**
- **Cardiovascular,**
- **Neurological (AD, PD),**
- **Diabetes…**

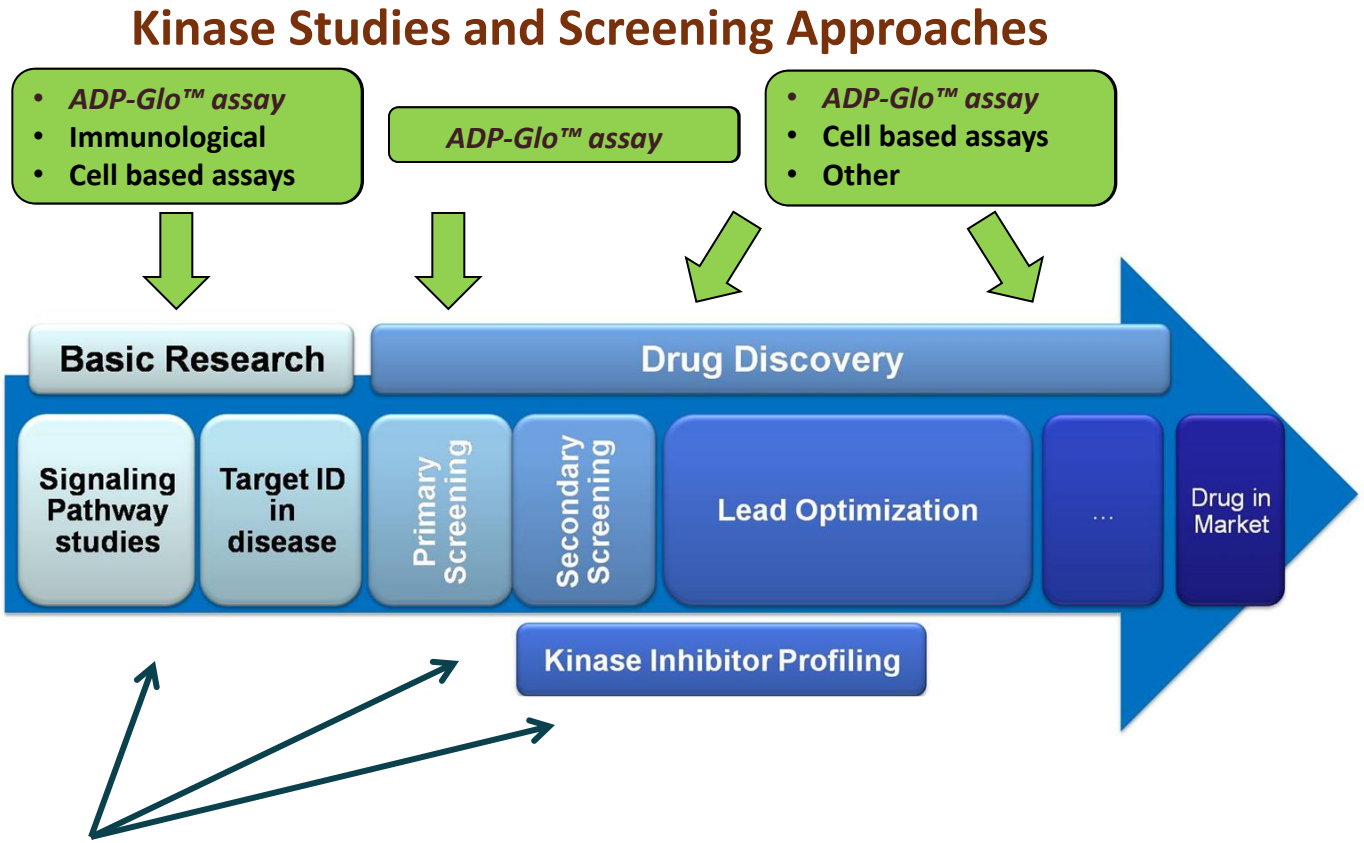
McDermott, U., Downing, J. R. and Stratton, M. R. (2011) *N Engl J Med* **364**, 340-50.

All Kinase Inhibitors are not equal
(Different trends of screenings)

- ATP competitive (low selectivity)
- ATP non competitive or Allosteric (more selective)
- DFG out inhibitors (unactive form, more selective)
- DFG in inhibitors (active form)
- Type 1 ½ (Back pocket of ATP site, improve selectivity)
- DFG in after HTS then optimize to DFG out



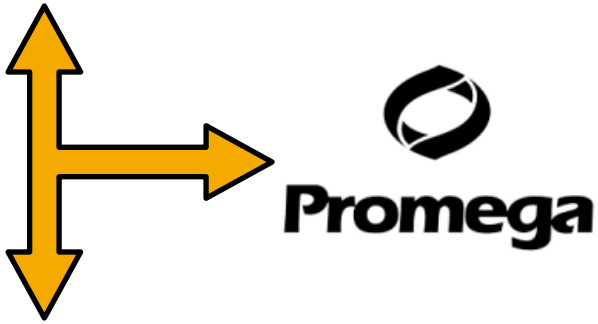
Kinase Studies in Basic Research and Drug Discovery



Need for a Universal Kinase Assay that can be applied to all types of Kinase Studies

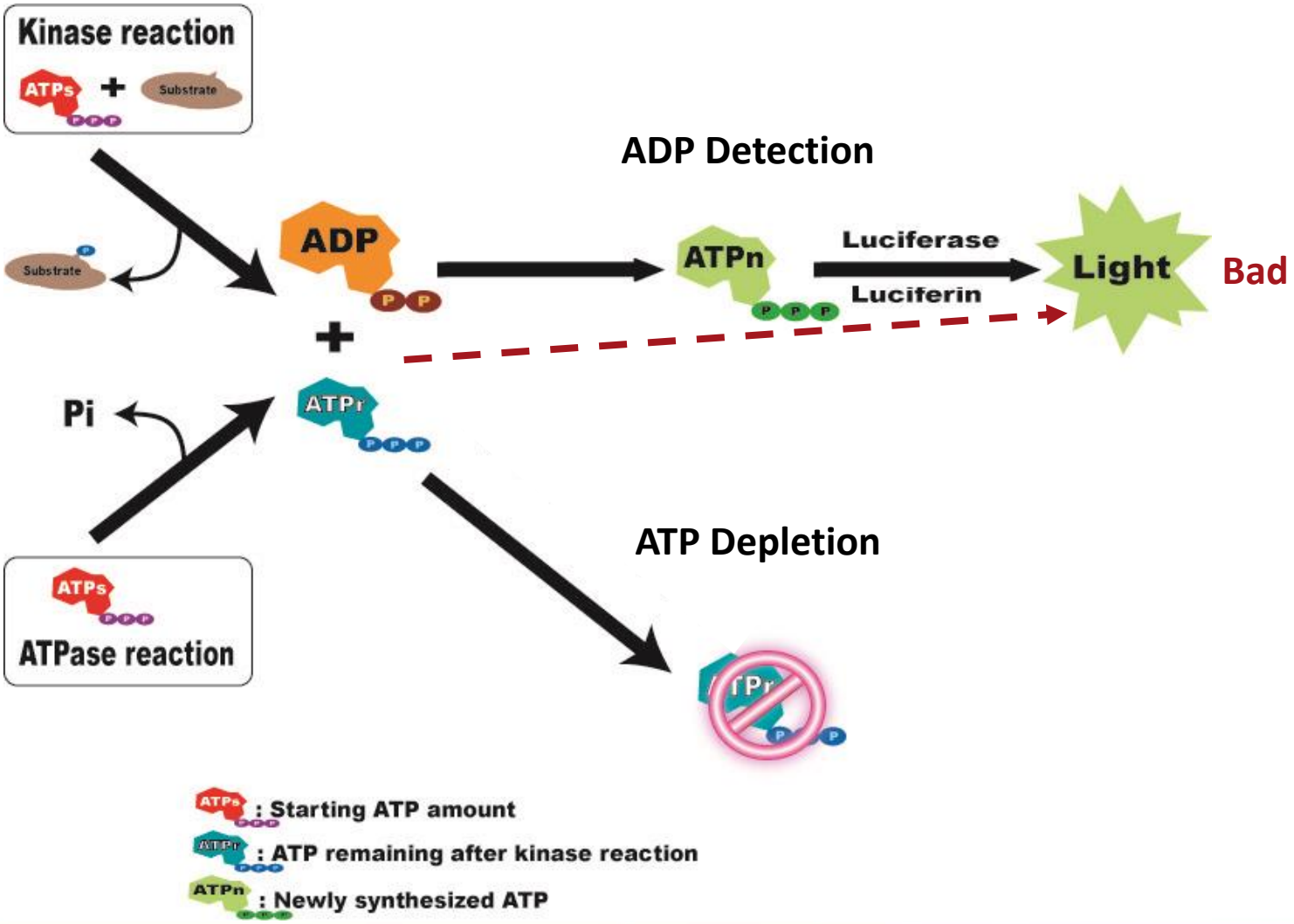
Kinase Research: parts of the equation

- Kinase families (targets)
- Kinase Assays (ADP-Glo)
- Kinase Inhibitors (Drugs)



Pharmaceuticals

Luminescent ADP detection assay principle



ADP-Glo Assay Format- 1:1:2

384-well plate

5µl kinase reaction

+

5µl ADP-Glo Reagent

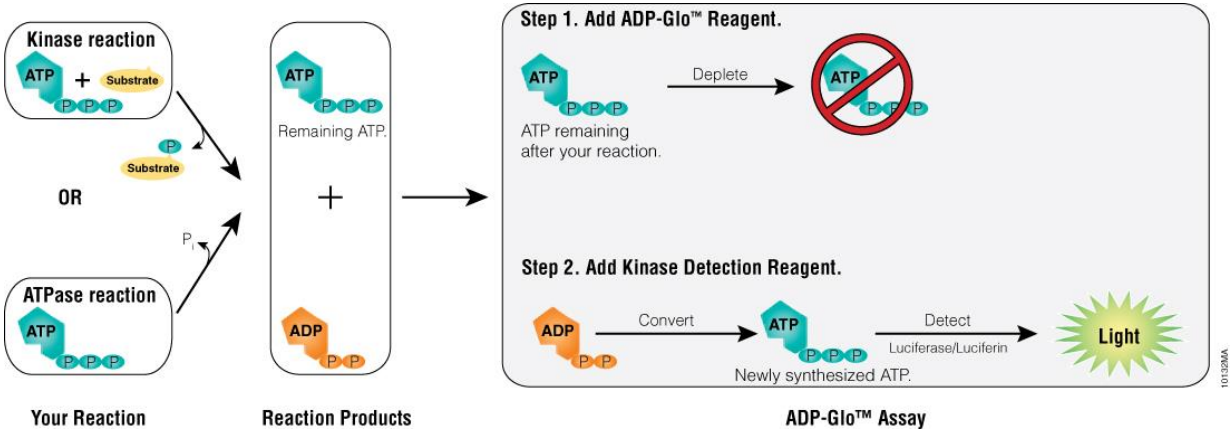
40 min. Incubation

+

10µl Kinase Detection Reagent

30-60 min. Incubation

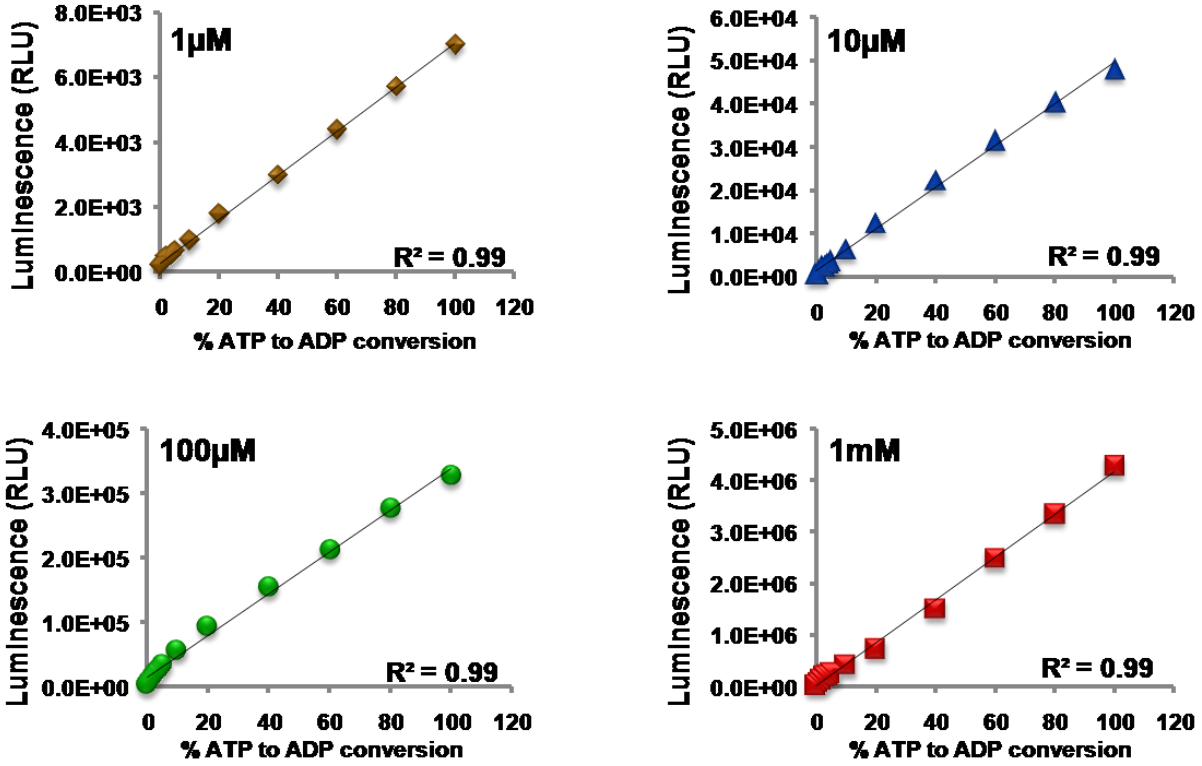
Record Luminescence



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

Linearity of the ADP-Glo assay

ADP conversion curves at different ADP/ATP concentrations



ADP-Glo can be used virtually with any ATP concentration

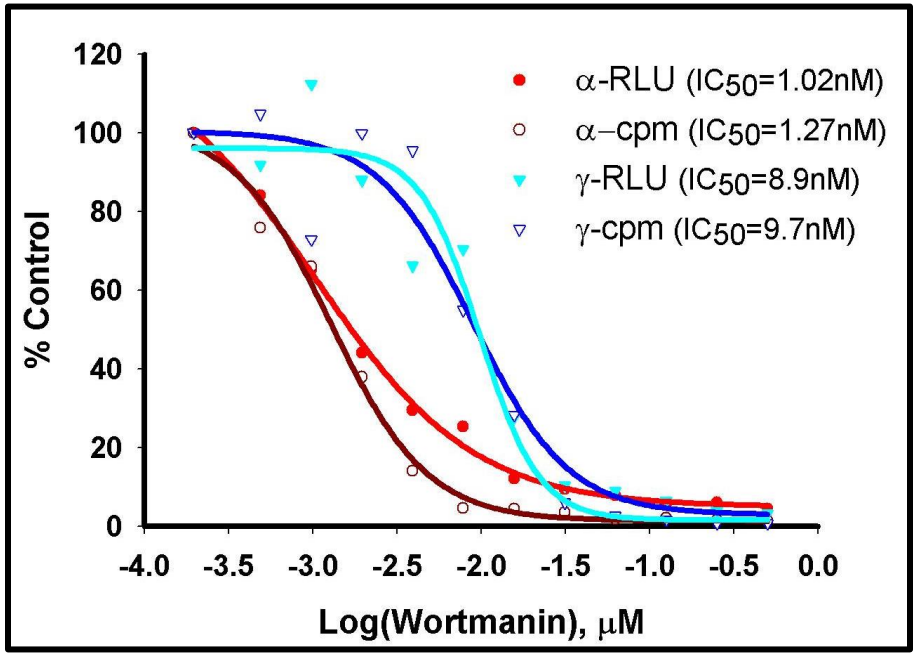
Sensitivity of the luminescent ADP detection assay

ADP-Glo™ Assay Performance Using Four Different Sources of ATP.

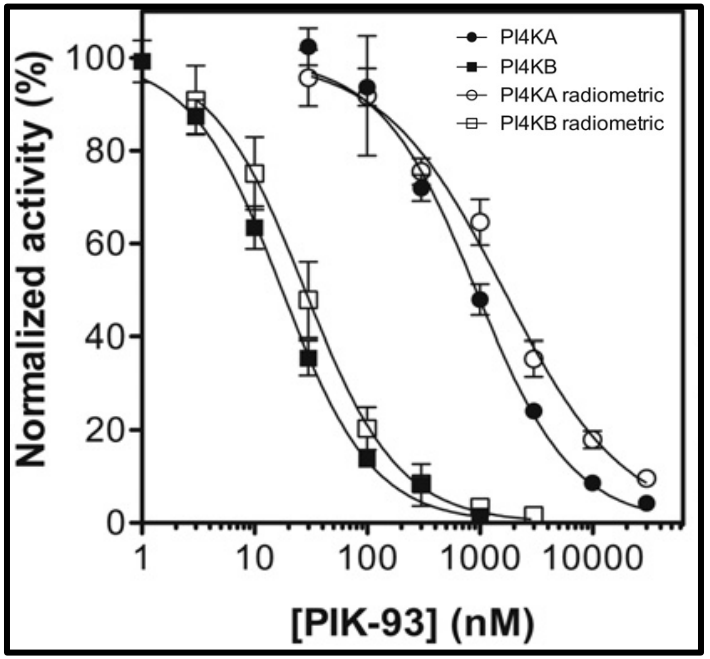
	ATP-ADP ranges								
	1mM			100µM			10µM		
% ADP in ATP + ADP mixture	20%	10%	5%	20%	10%	5%	20%	10%	5%
Promega	45	23	13	54	32	18	37	20	10
Competitor S	26	13	8	35	21	11	31	16	8
Competitor T	13	7	4	15	10	5	16	9	5
Competitor G	12	6	4	16	10	6	18	10	6

ADP-Glo sensitivity is a proven fact

Comparison between ADP Glo and Radioactivity Assay



Vidugiriene J, et al (2009). Evaluating the utility of a bioluminescent ADP-detecting assay for lipid kinases. Assay Drug Dev Technol. 7(6):585-97.

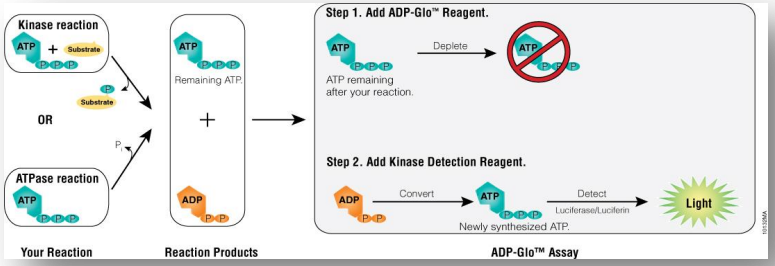


Tai AW, Bojjireddy N, Balla T. (2011) A homogeneous and nonisotopic assay for phosphatidylinositol 4-kinases. Anal Biochem. 417(1):97-102.

Specific activity and response to known inhibitors determined by ADP-Glo correlated well with data from radiometric assay

One assay platform - many applications

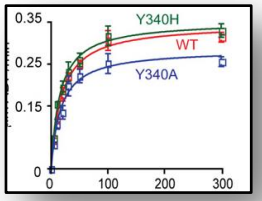
ADP-Glo is a Universal *in vitro* Biochemical Assay for all types of Kinase Studies



ADP-Glo Assay Platform

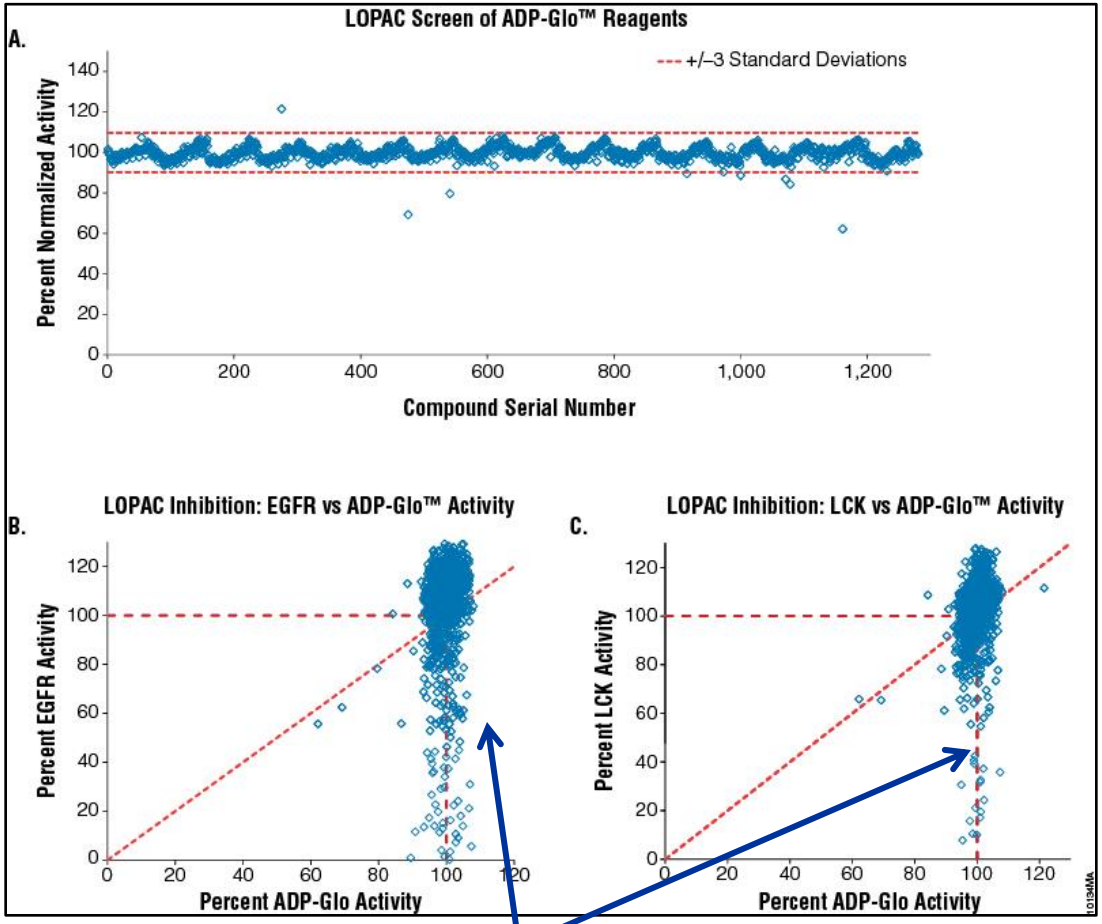
High-Throughput Screening

Mode of action studies



Kinase inhibitor profiling

ADP-Glo is resistant to chemical interference

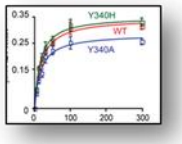


High-Throughput Screening

Identification of true Kinase inhibitors vs false positives using Luminescence Kinase assay

Determination of Biochemical Values Using ADP-Glo™ Assay

Mode of action studies



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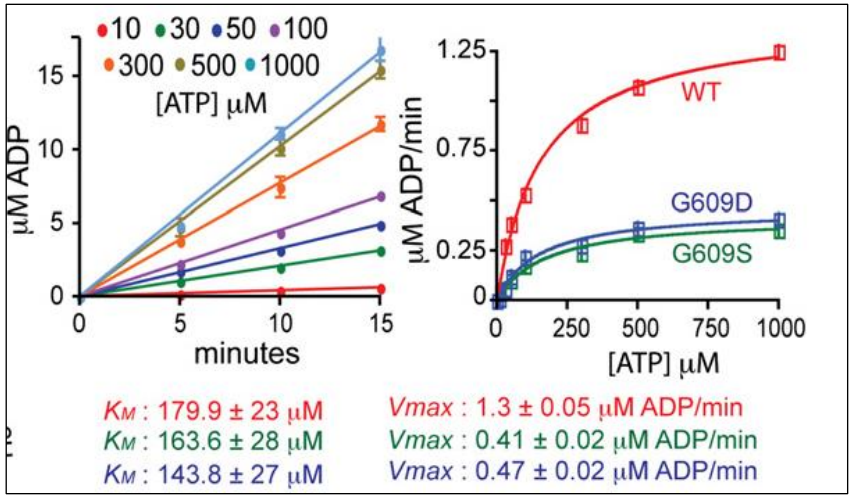
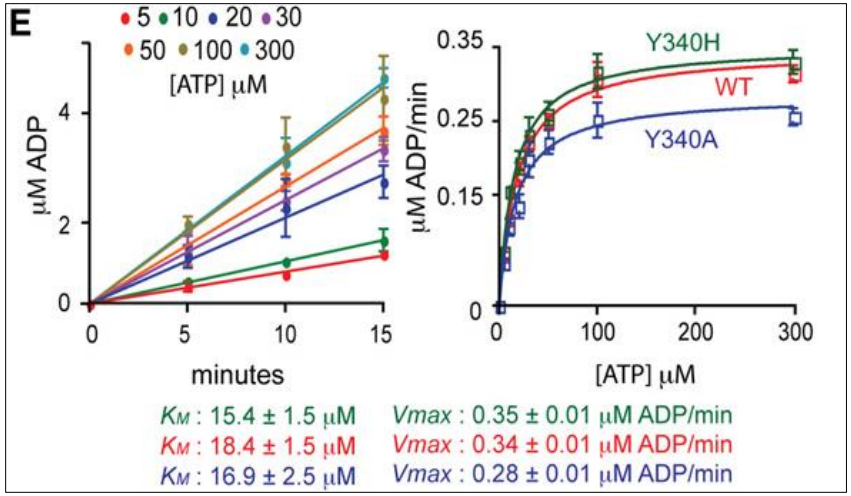


Haspin

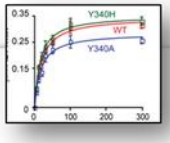


Balzano D et al., (2011). A general framework for inhibitor resistance in protein kinases. Chem Biol. 18(8):966-75.

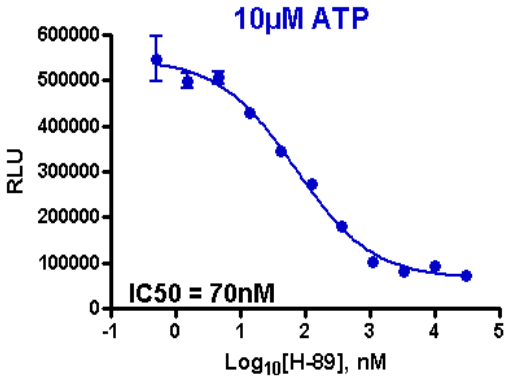
ADP-Glo™ Assay produces Km values similar to literature

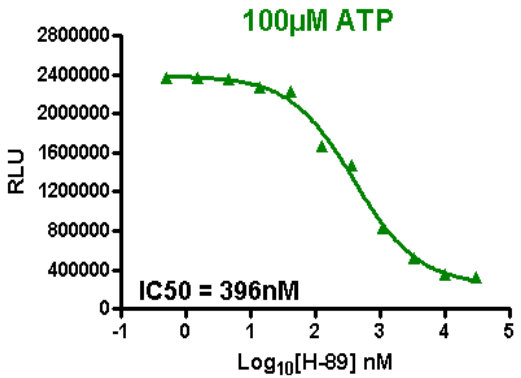
Determination of inhibitor's mechanism of action

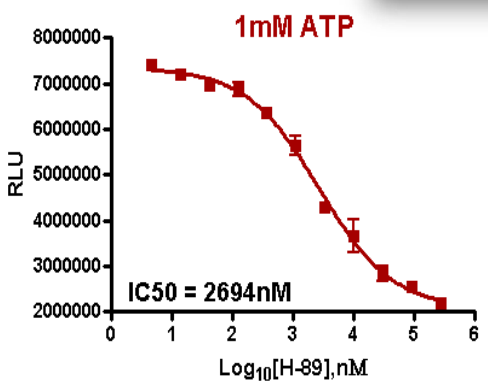
Mode of action studies



PKA ATP Competitive inhibitor H-89

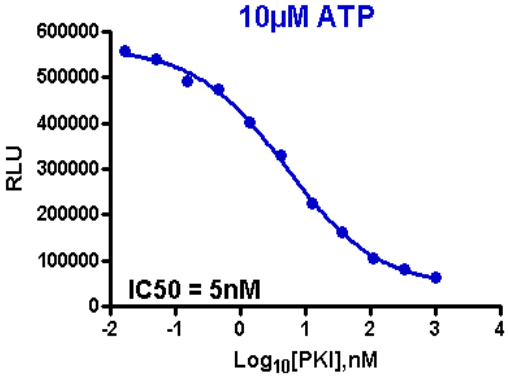


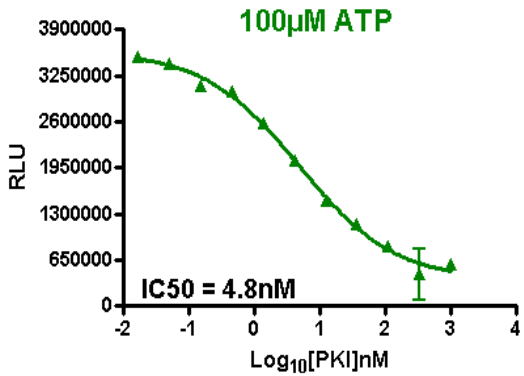


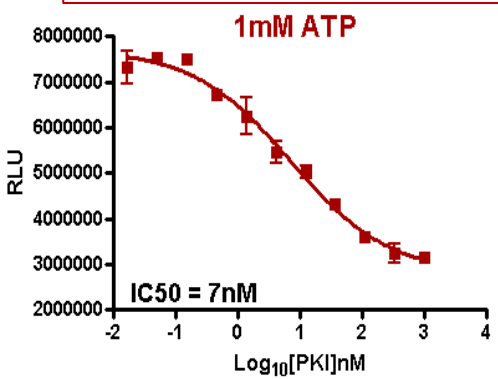


PKA ATP non Competitive inhibitor PKI

Cellular Levels mM







ADP-Glo is a perfect assay to distinguish between ATP competitive and non competitive kinase inhibitors

ADP-Glo Assay in peer review

A subset of ADP-Glo use citations for kinases (a full list is available upon request)

***A general framework for inhibitor resistance in protein kinases*.**
Balzano *et al.* Chemistry & Biology (2011) 18(8):966-975

Evidence that Aurora B is implicated in spindle checkpoint signaling independently of error correction.
Santaguida *et al.* The EMBO Journal (2011) 30, 1508-1519

Comparison of luminescence ADP production assay and radiometric scintillation proximity assay for cdc7 kinase.
Takagi *et al*. *Combinatorial Chemistry & High Throughput Screening (2011) 14(8):669-687*

A homogeneous and nonisotopic assay for phosphatidylinositol 4-kinases
Tai *et al*. Anal Biochem. (2011) 417(1):97-102

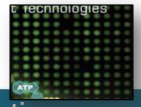
Deoxycytidine kinase regulates the G2/M checkpoint through interaction with cyclin-dependent kinase 1…
Yang *et al.* Nucleic Acid Research 2012, 40(19):9621-9632

Development and Validation of a High-Throughput Intrinsic ATPase Activity Assay for the Discovery of MEKK2…
Ahmad *et al.,* J Biomol Screen. 2012 Nov 7

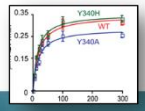
Domain-Based Biosensor Assay to Screen for Epidermal Growth Factor Receptor Modulators in Live Cells
Antczak *et al.,* ASSAY and Drug Development Technologies 2012, 10(1): 24-36.

STK33 kinase inhibitor BRD-8899 has no effect on KRAS-dependent cancer cell viability
Luo *et al.,* PNAS, 2012 vol. 109 (8), 2860-2865 .

High-Throughput Screening



Mode of action studies

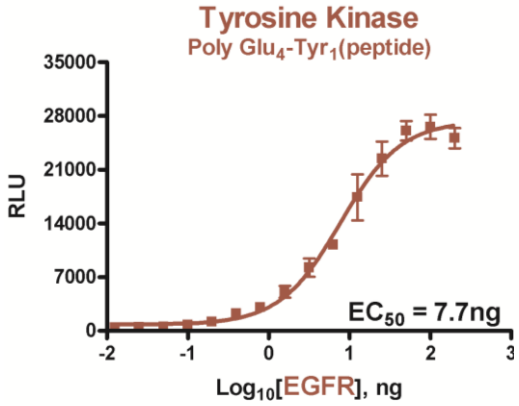


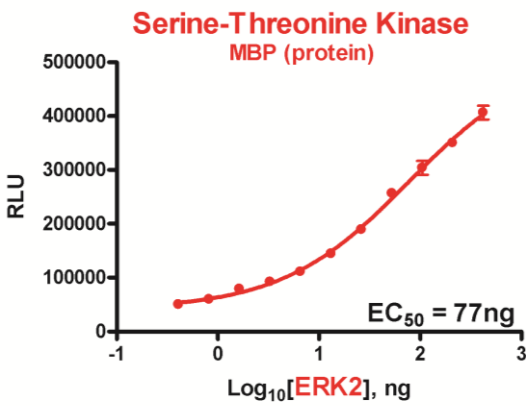
Kinase inhibitor pr[illegible]ng

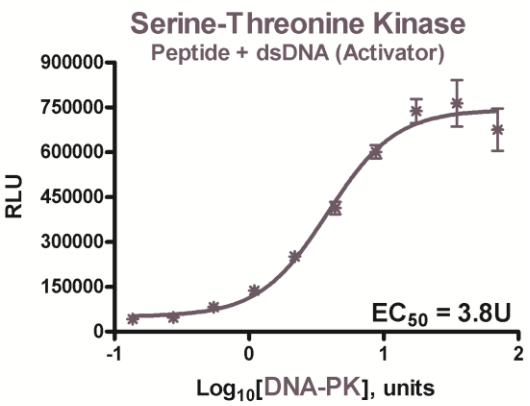
Diverse kinase-substrate combinations with ADP-Glo

Kinase Inhibitor Profiling

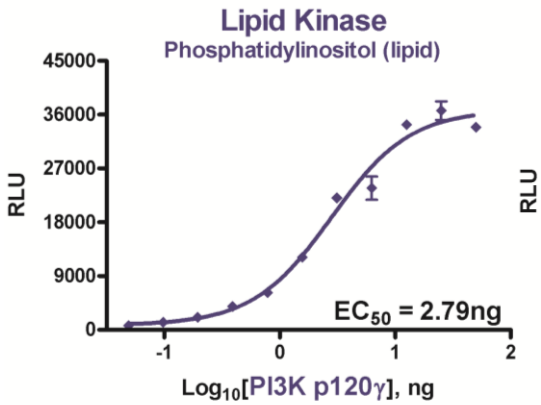
Protein Kinases

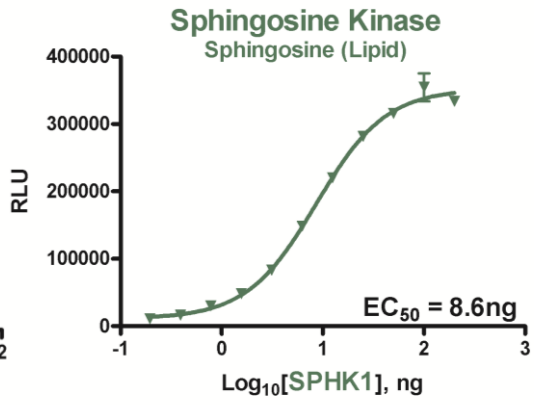


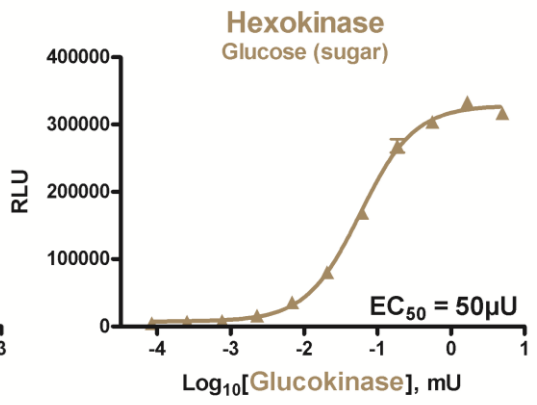




Non-Classical Kinases

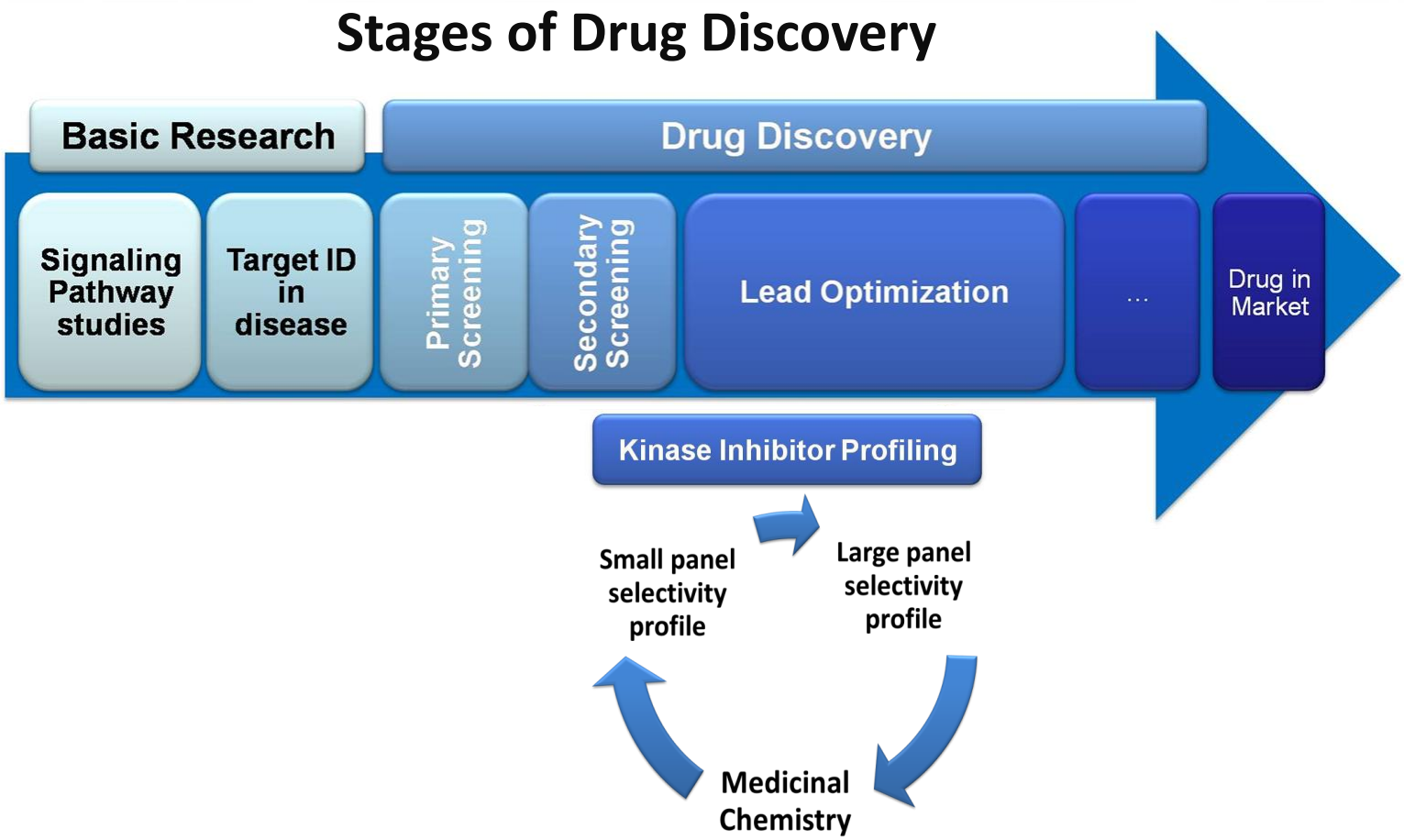






ADP-Glo Kinase Assay detects the activity of any Kinase regardless of the substrate chemical structure

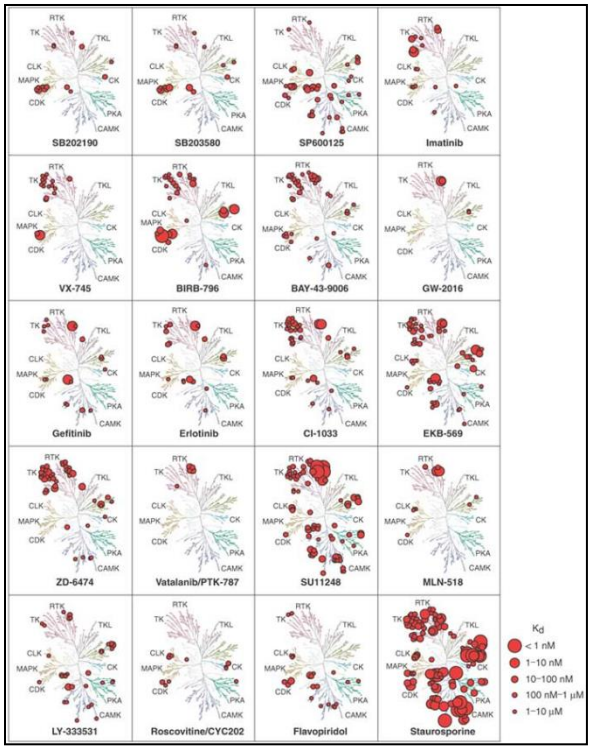
Kinase inhibitor Profiling is an important Step of Drug Discovery

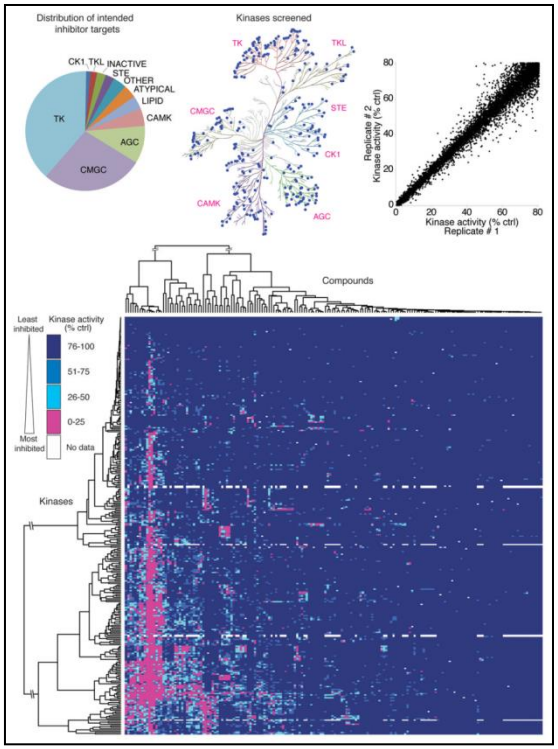


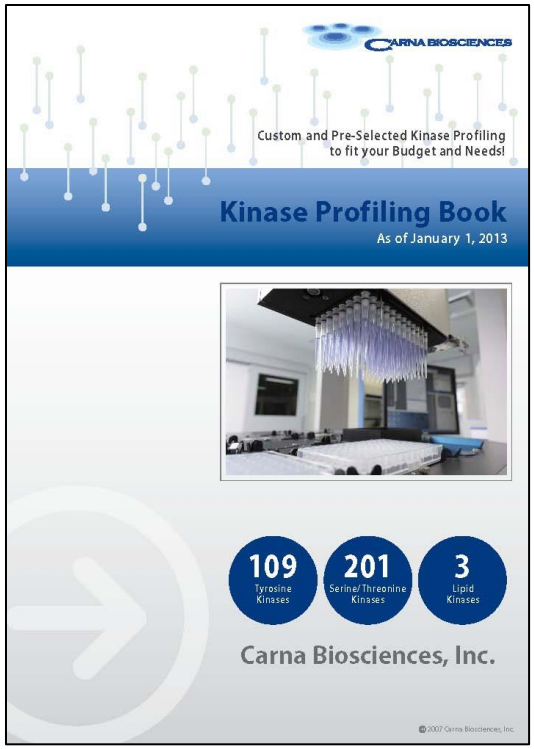
Kinase selectivity profiling allows for a balance between potency and selectivity of lead compounds during drug discovery

Kinase Profiling data generated in many ways

Many profiling platforms exist already







Binding **Radiometric** **Mobility Shift**

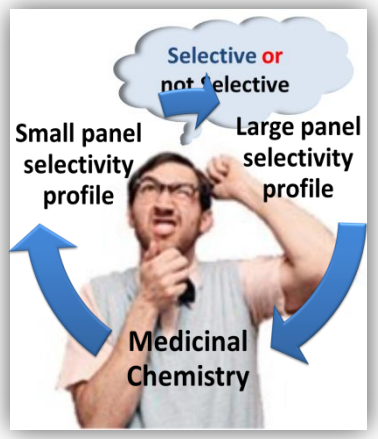
Large panel kinase profiles may not be performed routinely

Choosing the right approach for best results

Issues related to Kinase Profiling

Current Challenges in creating selectivity profiles for Kinase inhibitors

- ✓ **Addressing confidentiality issues related to outsourcing.**
- ✓ **In house profiling requires kinase assay developments and rigorous optimization which can be time consuming.**
- ✓ **The lack of having enough enzymes available to do the profiling.**
- ✓ **Generating Selectivity profiles is costly and time consuming.**
- ✓ **Immediate accessibility of the data upon completion of Profiling.**



There is a need in an easy and flexible solution that allows kinase selectivity profiles to be generated routinely without the time and cost challenges.

Kinase Enzyme System (KES) format

Promega

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

SignalChem
Specialists in Signaling Proteins

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

KES: a complete kinase solution

Lipid Kinase Systems

Complete solution for measuring PI kinases without the use of radioactivity, lipid substrate modification or lipid extraction

PI3K class I enzymes:

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

+

PI:PS Lipid Kinase substrate 0.5ml x 1mM

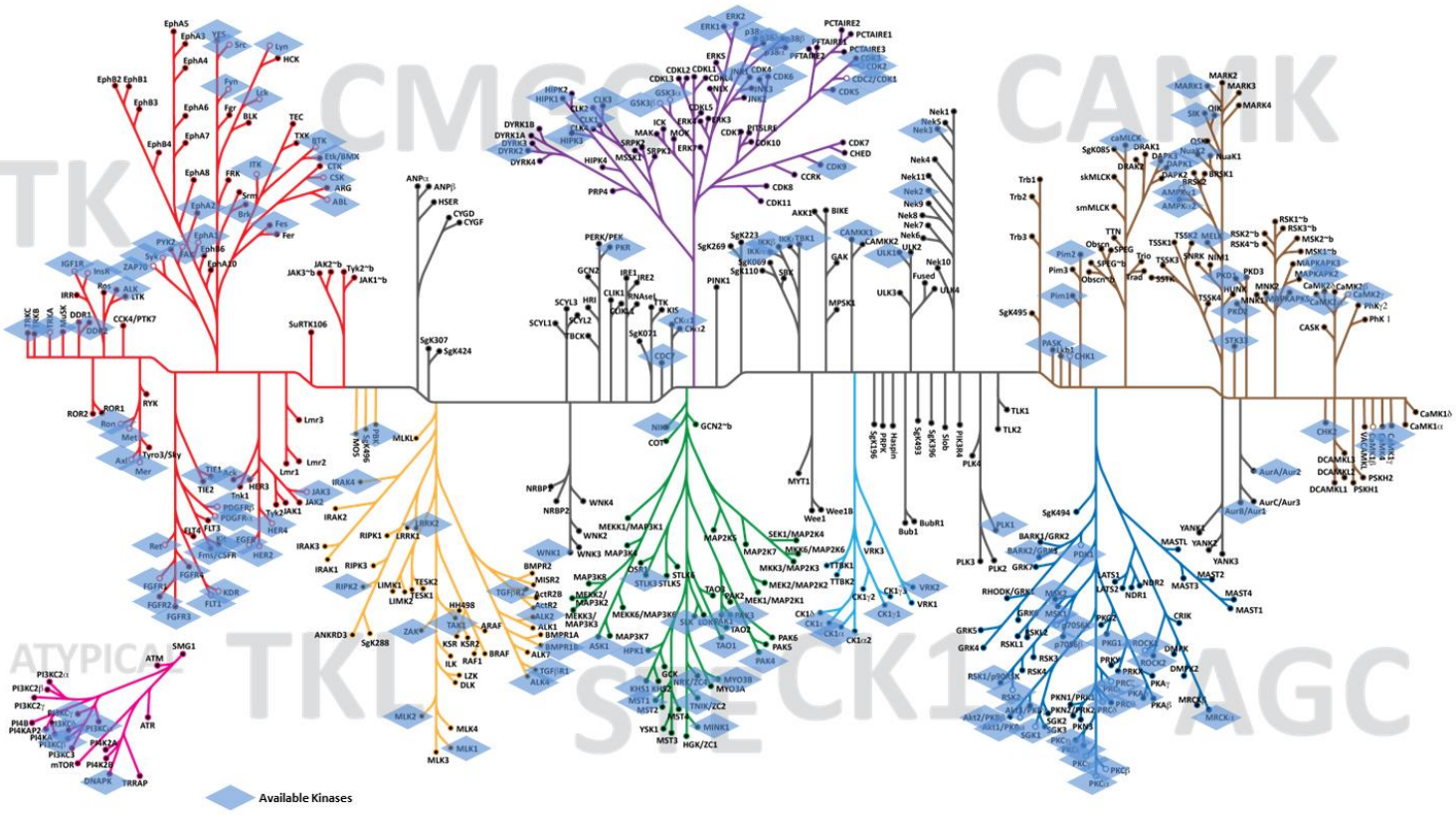
or

PIP2:PS Lipid Kinase substrate 0.25ml x 1mM

ADP-Glo Kinase Assay

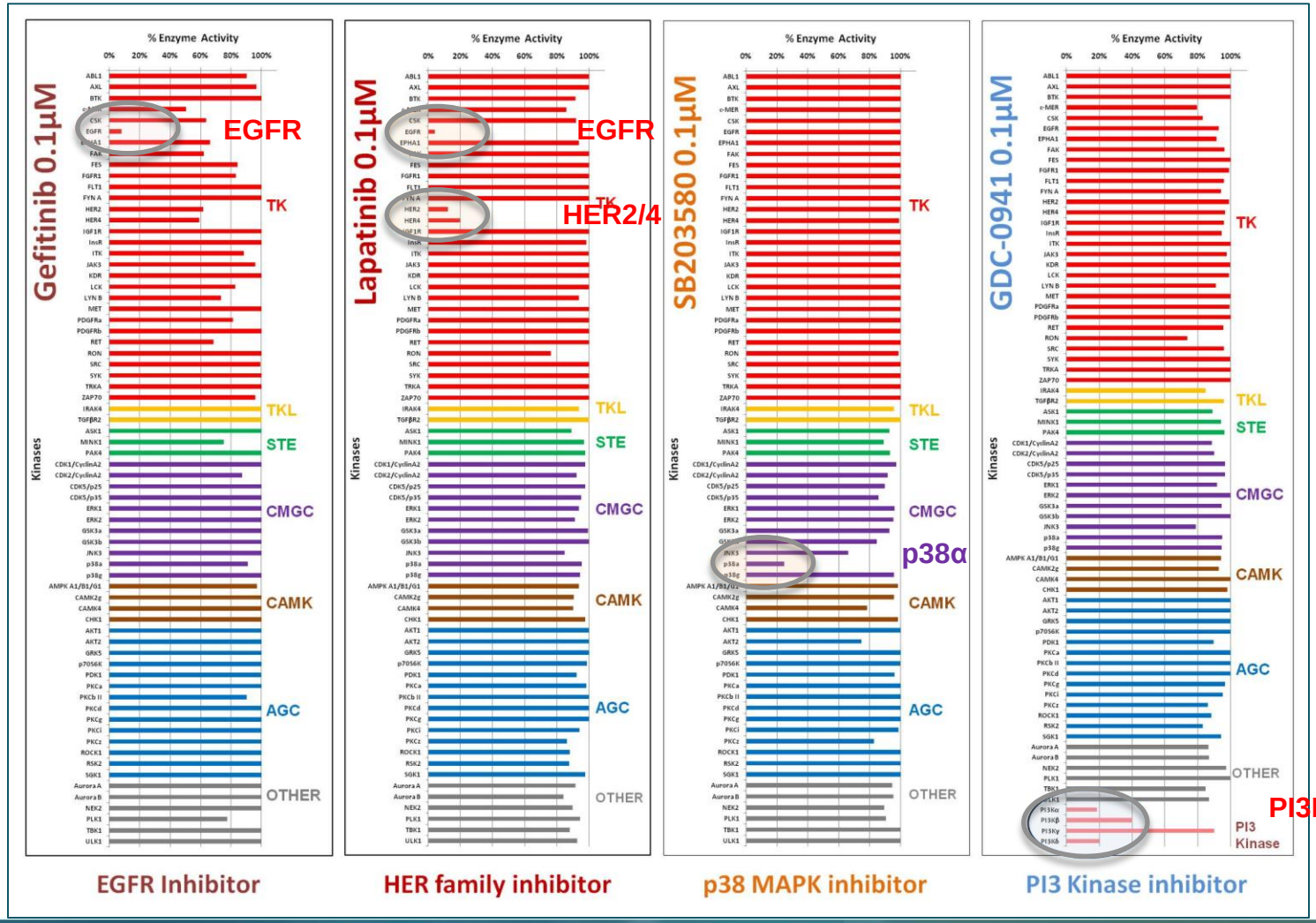
Addressing the kinome with large kinase panel

Profiling Panel should include close and distant kinases to assess compound selectivity



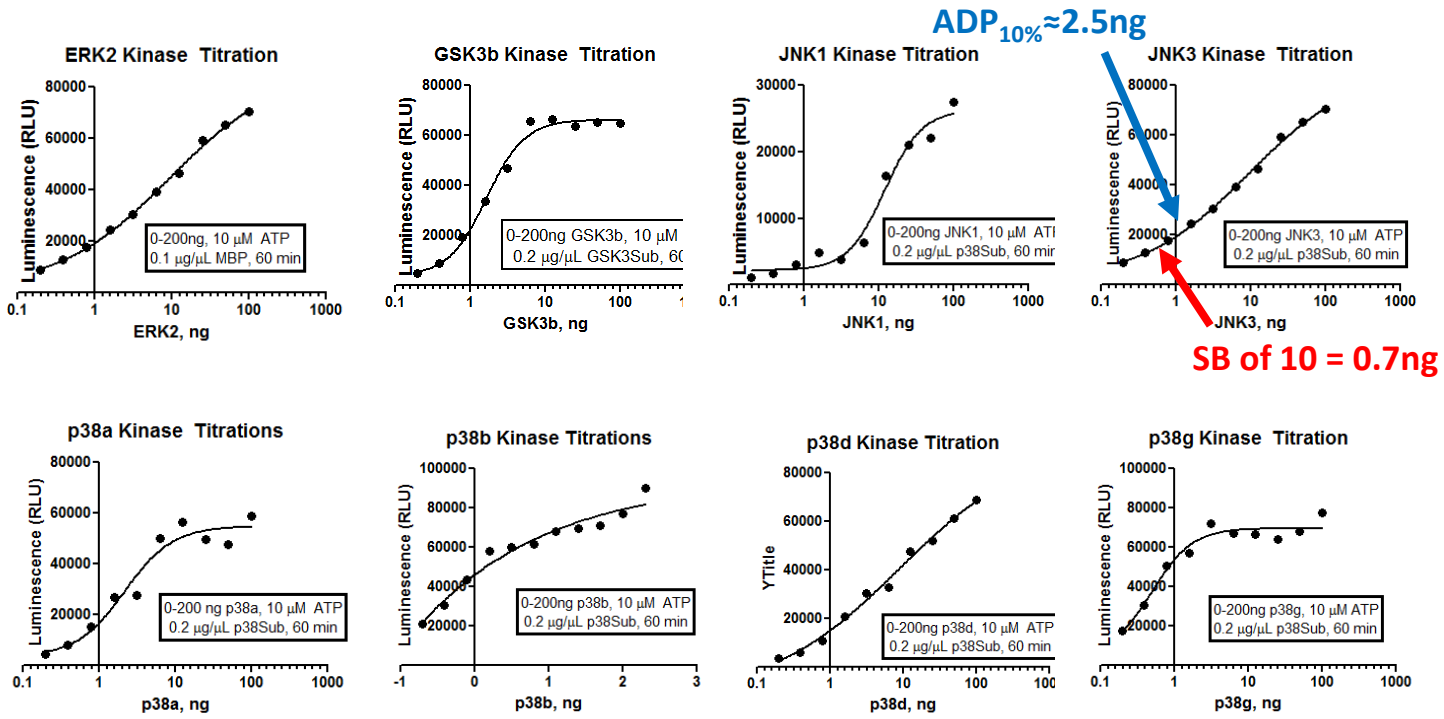
Broad Human Kinome coverage with >170 Kinase Enzyme Systems

Profiling inhibitors against a subset Kinase panel with ADP-Glo Kinase Assay



Optimizing enzyme concentration for kinase inhibitor studies

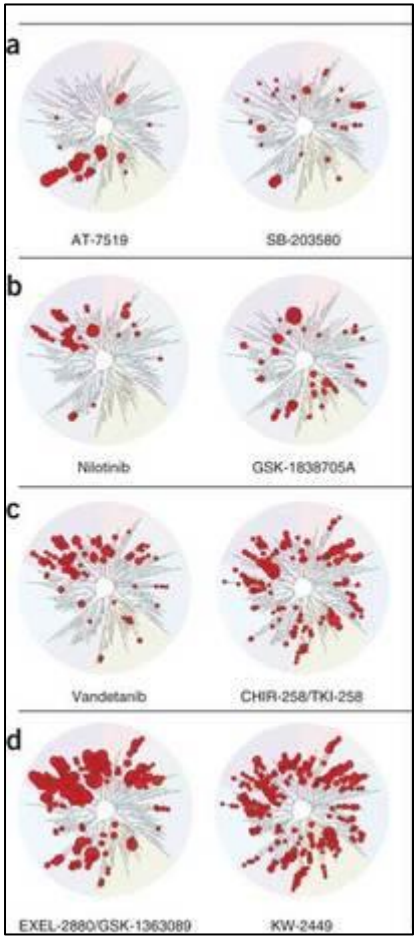
kinases need to be titrated to find the optimal amount to be used

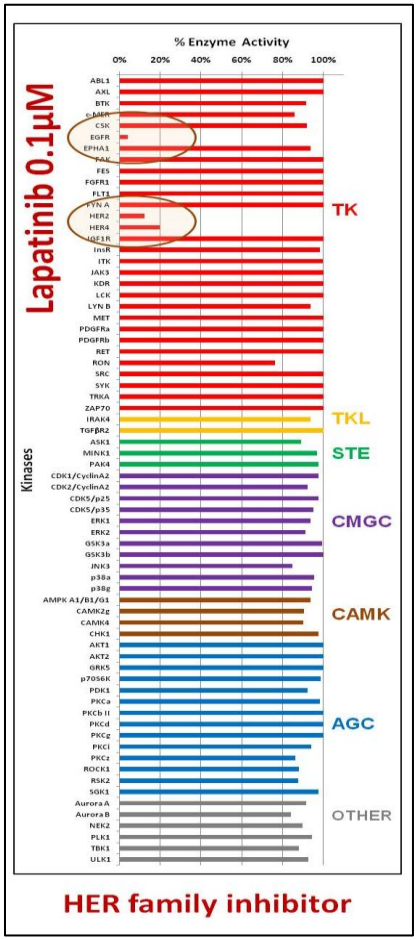


➢**Determination of SB and % conversion values:** kinase amount needed to generate a signal-to-background ratio of >=10 and optimal % ATP conversion.

Large kinase profiling data vs. routine small selectivity panels

Did we achieve our goal of ease of use and flexibility?





- ✓ **Costly and time consuming**
- ✓ **Extensive assay development required.**

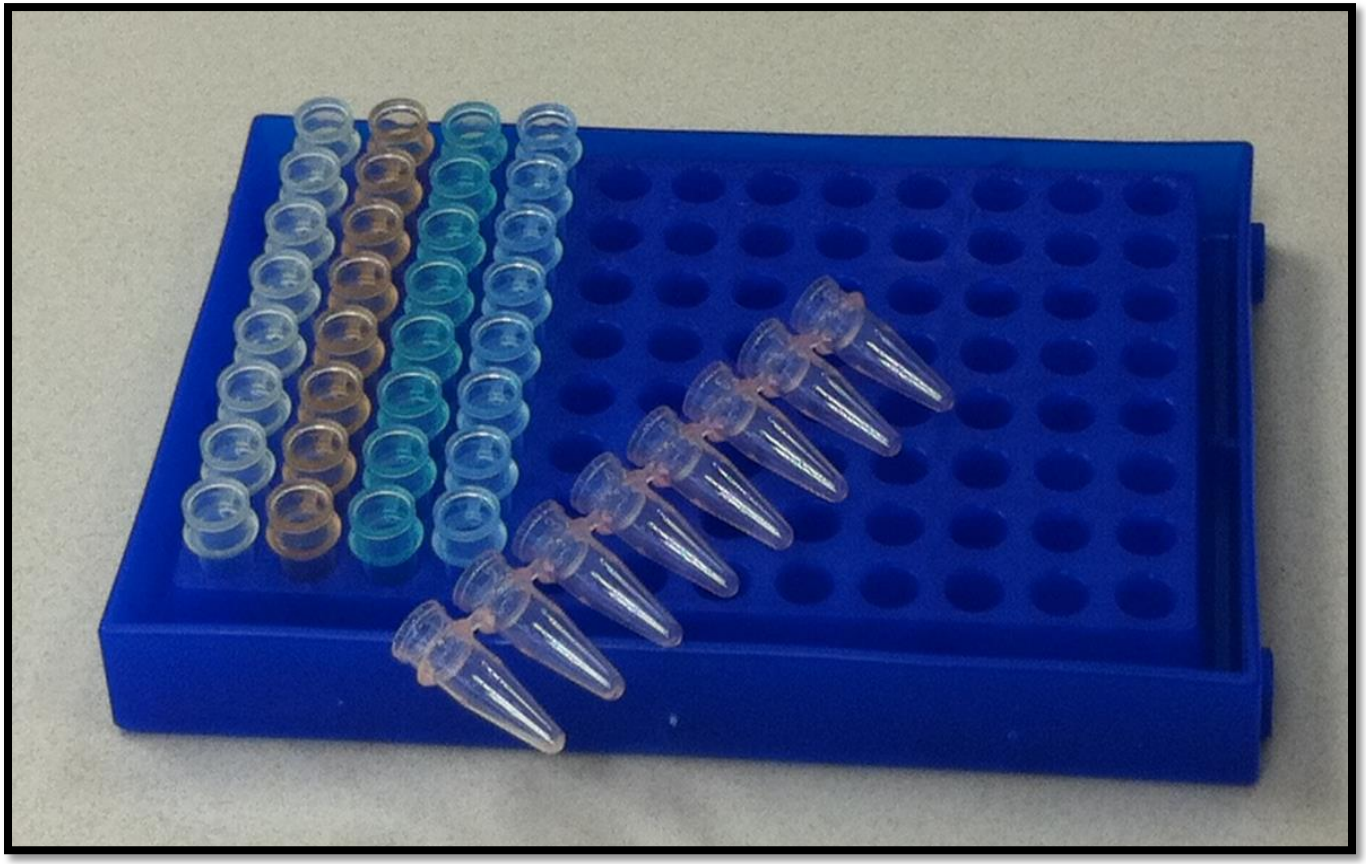
Developing a simple kinase profiling assay

Choosing the right kinases for flexible and targeted inhibitor profiling

17 Kinase strips (112* Kinases)

***14 Family groups and 3 mixed panels**

Important kinase targets organized by kinase families in 8 well strips



- **Ready to profile with simple protocol (No development time required)**
- **Cost effective**
- **Quick results with no compound sending out**

Kinase profiling Systems: 2 types

Family based strips

TK-1	TK-2	TK-3	TK-4	CMGC-1	CMGC-2	TKL-1	STE-1	CAMK-1	CAMK-2	AGC-1	AGC-2	OTHER/CK-1	OTHER-2
EGFR	ABL1	AXL1	c-MER	ERK2	CDK1/CyclinA2	ALK2	ASK1	CHK1	AMPK A1/B1/G1	AKT1	PKCa	Aurora A	CDC7/DBF4
HER2	BRK	EPHA1	FGFR1	GSK3b	CDK2/CyclinE1	ALK4	HPK1	CHK2	AMPK A1/B1/G2	p70S6Kb	PKCb II	Aurora B	EIF2AK2
HER4	BTK	FAK	FGFR2	JNK1	CDK3/CyclinE1	IRAK4	MINK1	MAPKAPK2	AMPK A2/B1/G1	PDK1	PKCd	CK2a1	IKKb
IGF1R	CSK	ITK	FGFR4	JNK3	CDK5/p25	MLK2	MST1	MARK1	CAMK2a	PKA	PKCe	DNA-PK	NEK2
InsR	FYN A	JAK3	FLT1	p38a	CDK5/p35	RIPK2	NIK	MELK	CAMK2g	PKC	PKCg	CK1α1	NEK3
KDR	LCK	PYK2	FMS	p38b	CDK6/Cyclin D3	TAK1-TAB1	PAK1/CDC42	PASK	CAMK4	PRKG1	PKCi	CK1epsilon	PLK1
PDGFRa	LYN B	SYK	MET	p38d	CDK9/Cyclin K	TGFbR2	PAK3	PIM1	DAPK1	ROCK1	PKCtheta	CK1g1	TBK1
PDGFRb	SRC	TRKA	RET	p38g	CLK3	ZAK	TNIK	PKCmu (PKD1)	STK33	RSK2	PKCz	VRK2	ULK1

General panel

General Panel		
FGFR1	CDK2/CyclinE1	AKT1
JAK3	GSK3b	PKCa
LCK	p38a	ROCK1
SYK	AMPK A1/B1/G2	Aurora A
MINK1	CAMK4	CK2a1
PAK1/CDC42	CHK1	IKKb
IRAK4	DAPK1	CK1a1
TAK1-TAB1	MAPKAPK2	CK1g1

Relevant kinases organized in:

- **Family based strips for intra-family inhibitor promiscuity analysis.**
- **General panel for quick selectivity assessment.**
- **Multiple strips are used for large selectivity profiling.**

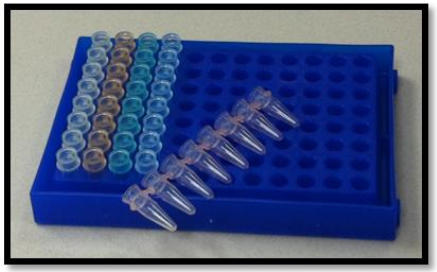
kinase profiling strip Concept

How does it work?

1	9	17	25	33	41	49	57	65	73	81	89
2	10	18	26	34	42	50	58	66	74	82	90
3	11	19	27	35	43	51	59	67	75	83	91
4	12	20	28	36	44	52	60	68	76	84	92
5	13	21	29	37	45	53	61	69	77	85	93
6	14	22	30	38	46	54	62	70	78	86	94
7	15	23	31	39	47	55	63	71	79	87	95
8	16	24	32	40	48	56	64	72	80	88	96

50x

Kinase Stocks (5µl)



S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

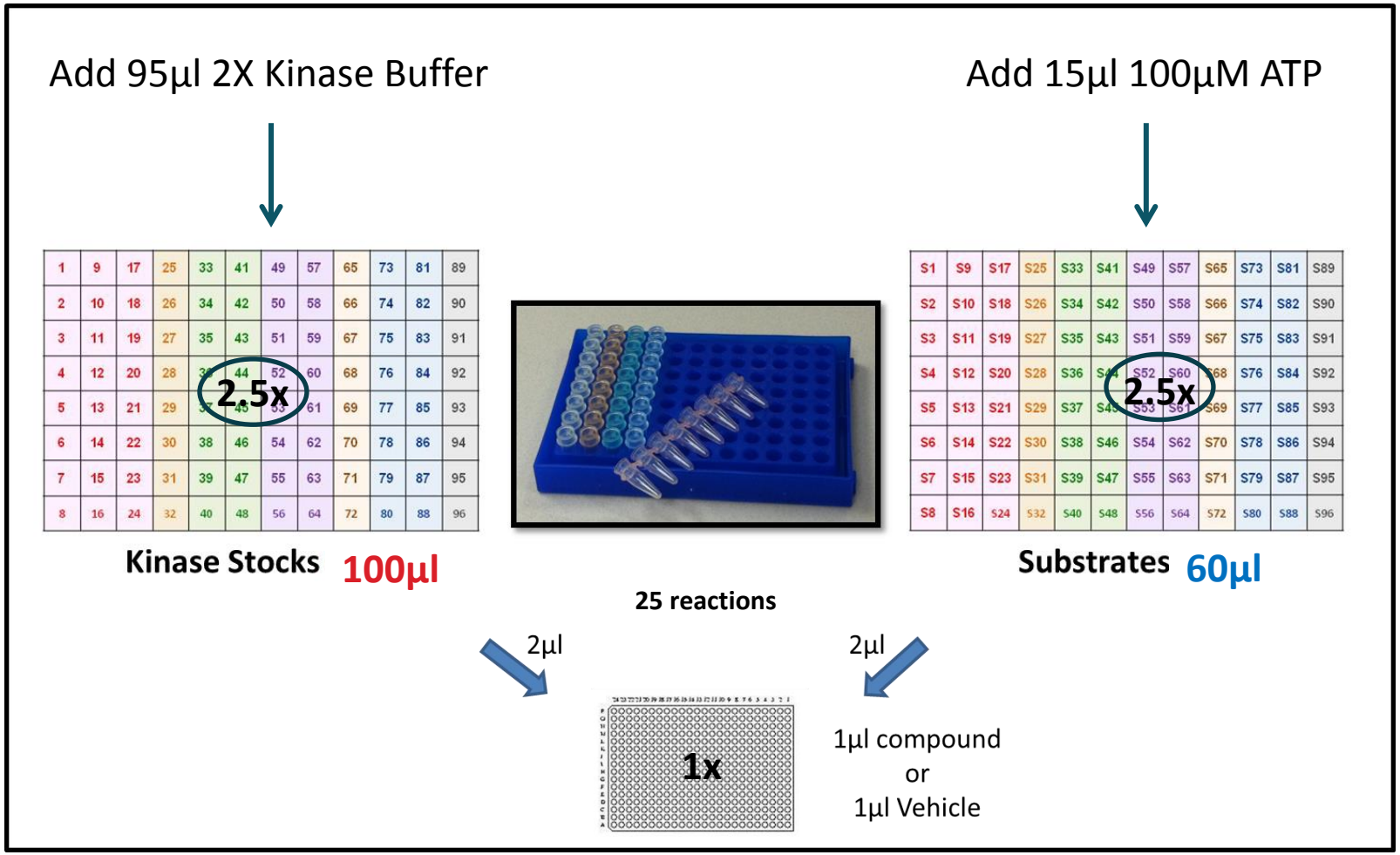
3.3x

Substrates (45µl)

Simple reaction assembly

kinase profiling strip Concept

How does it work?



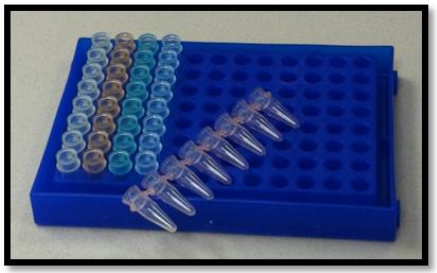
Simple reaction assembly

kinase profiling strip Concept

How does it work?

Add 95µl 2X Kinase Buffer

Add 15µl 100µM ATP



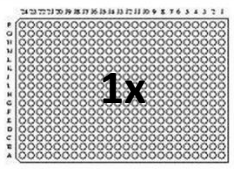
Kinase Stocks 100µl

Substrates 60µl

25 reactions

2µl

2µl



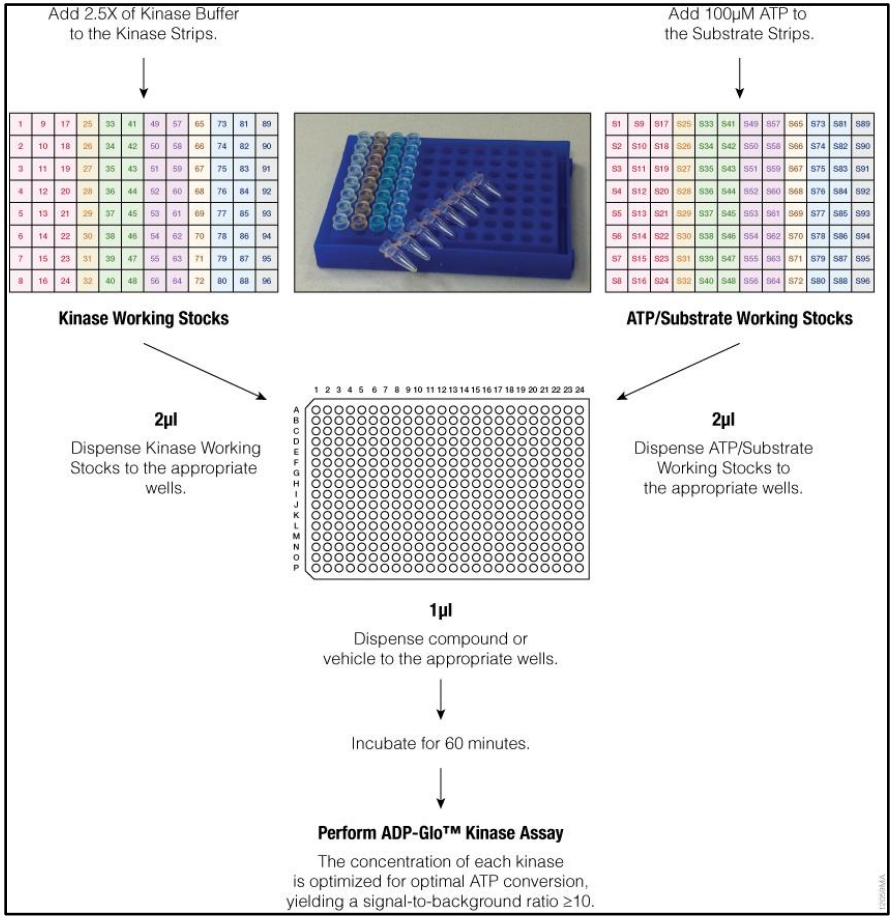
1µl compound
or
1µl Vehicle

Each enzyme will generate an optimal % ATP conversion with an SB>10 fold

Simple profiling protocol for flexible and targeted inhibitor profiling

Kinase Profiling Strip Systems:

- ✓ **One-time use design**: Eliminating multiple freeze/thaw cycles ensures optimal kinase activity per experiment.
- ✓ **Flexible kinase inhibitor profiling:** Each strip has enough material to profile:
  - **Dose response for 1 compound against the 8 kinases at once.**
  - **10 compounds at a single dose**
- ✓ **Easy-to-automate inhibitor profiling.**



Profiling with ADP-Glo platform made simple

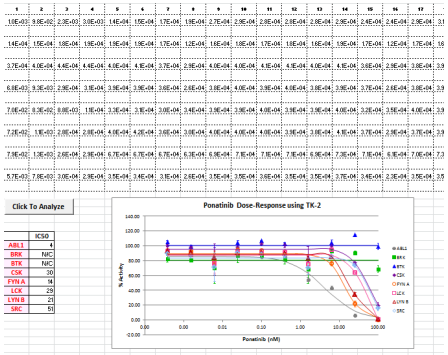
Streamline Your Kinase Profiling Workflow

Liquid Handling

Detection

GloMax®
Discover

Data Analysis



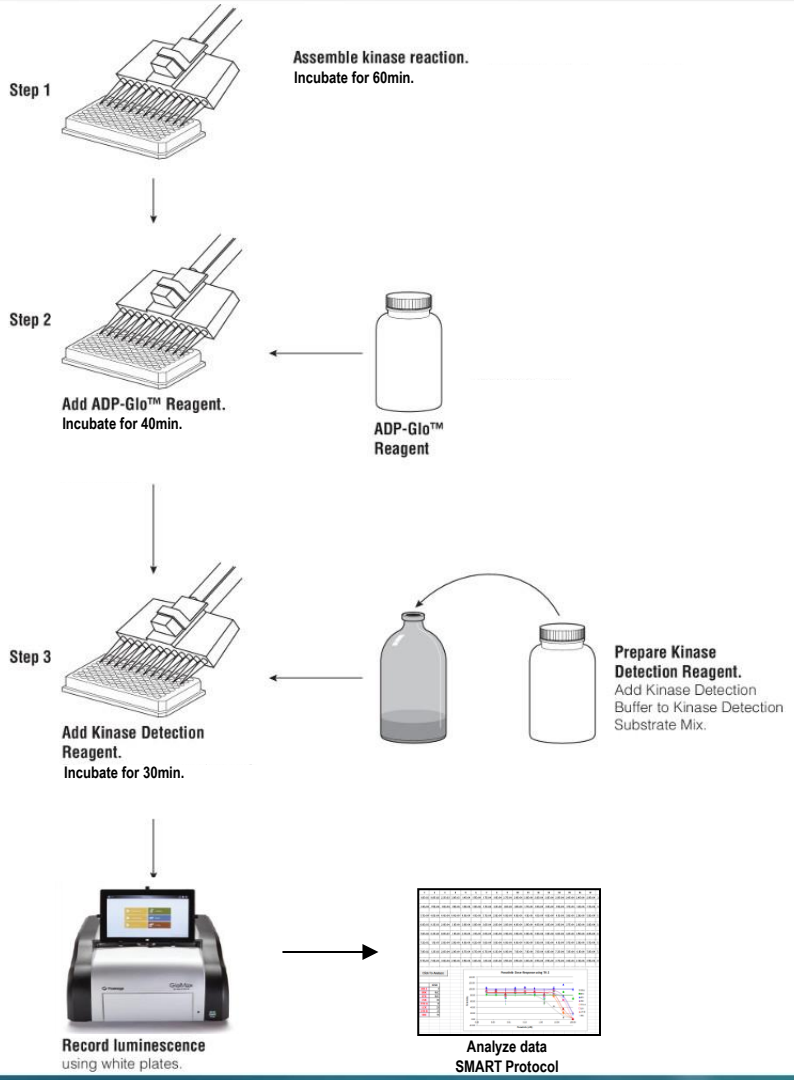
GloMax®
SMART Protocol

Automation of kinase profiling strip

How does it work?

Click on the link below to watch the video
https://p.widencdn.net/pwhnbo

ADP-Glo Assay Scheme



- **Two simple additions after the Kinase reaction**
- **Record luminescence and export automatically data to SMART Protocol**

Easily Collect Kinase Profiling Data and Quickly Analyze Results using GloMax® Systems

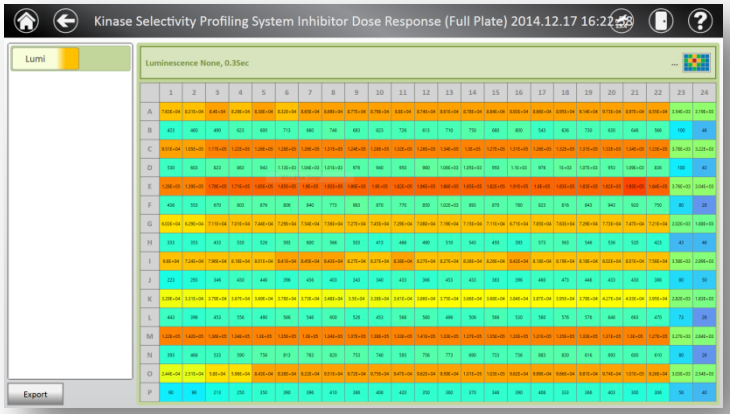
Select the built-in Kinase Selectivity Profiling SMART Protocol

- Screen kinase inhibitors
- Automatically analyze results

Over 50 Promega assay protocols are optimized and included with the GloMax® Discover System to streamline data collection and simplify your research

Easily Collect Kinase Profiling Data and Quickly Analyze Results using GloMax® Systems

View a heat map display to quickly asses the data



View analyzed dose response results after data collection

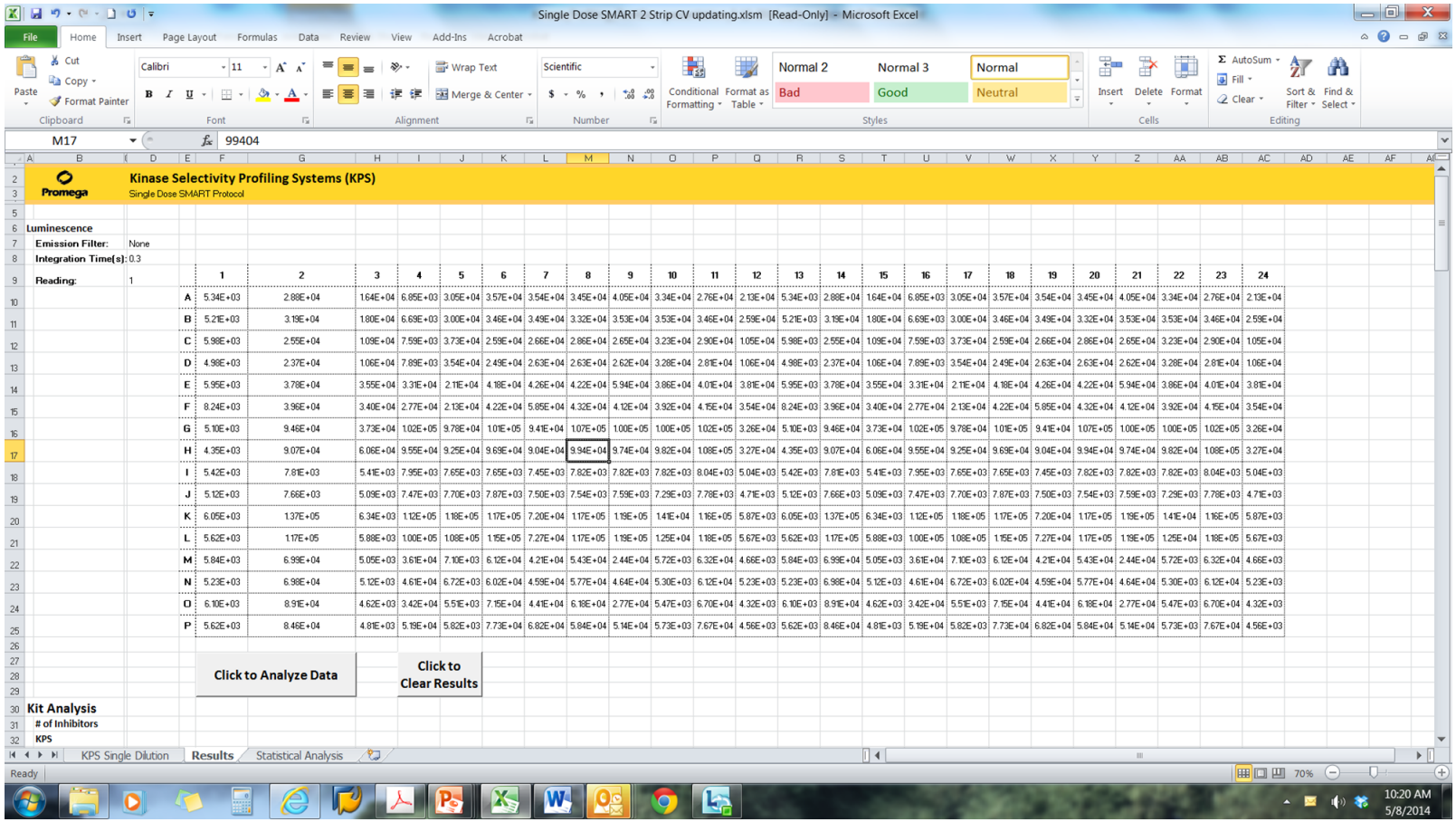
Kinase Selectivity Profiling Systems (KPS) *

Single Dose SMART Protocol

*Also available as a downloadable template on promega.com

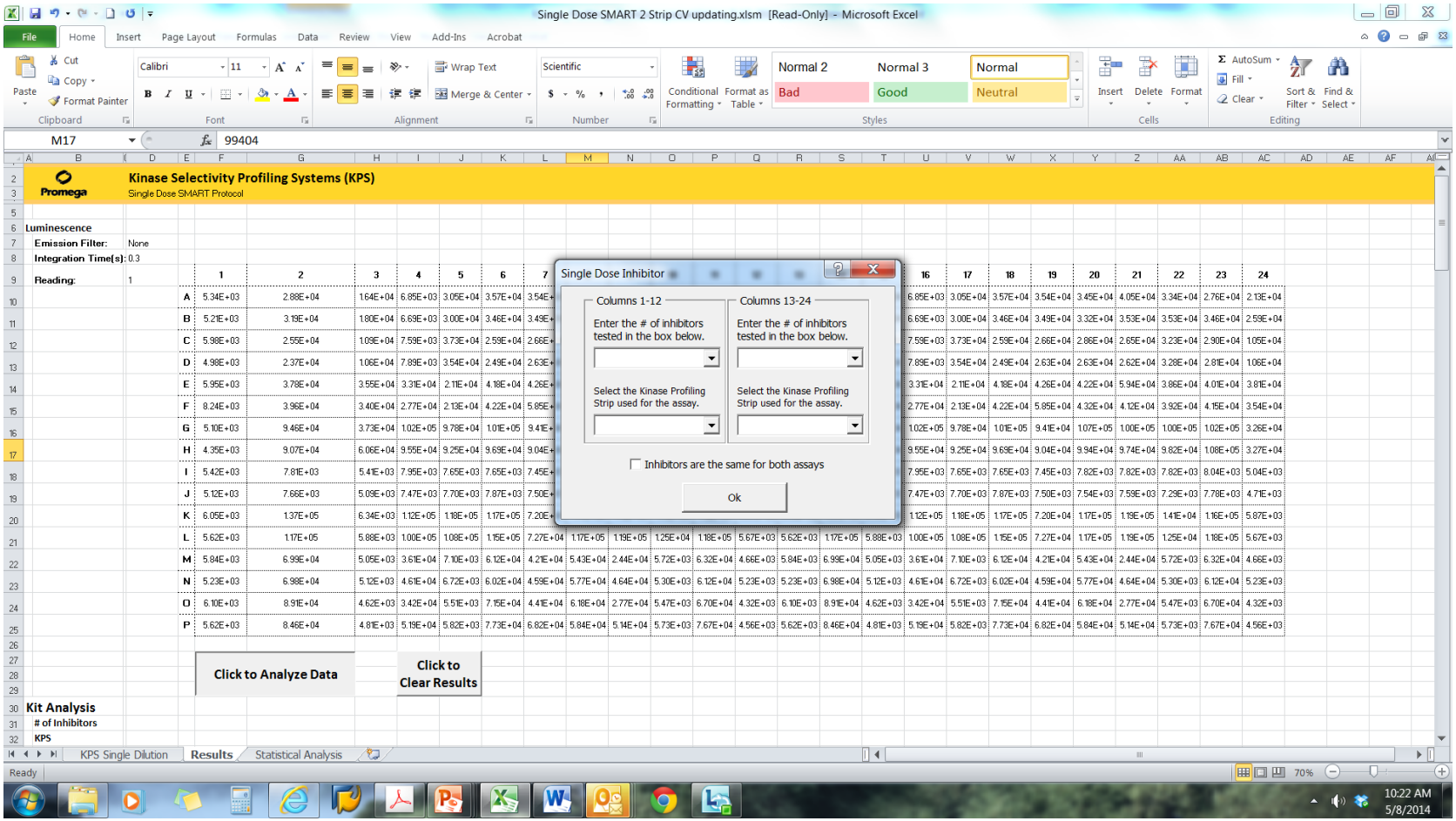
Single Dose SMART Protocol

Raw data is imported automatically in the template. Click to analyze.



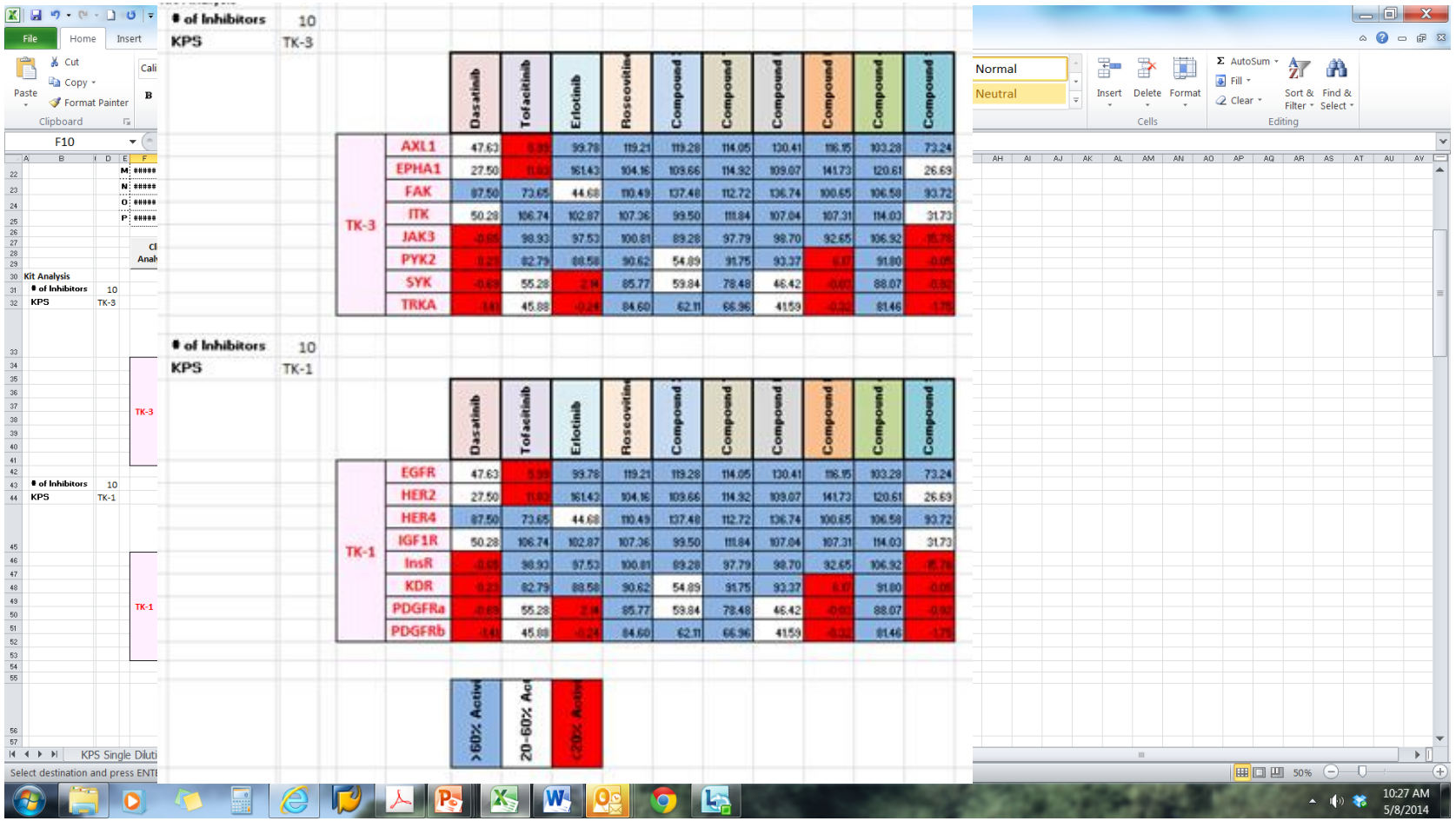
Single Dose SMART Protocol

Answer simple questions about the experiment: Strip kind, inhibitors, etc…



Single Dose SMART Protocol

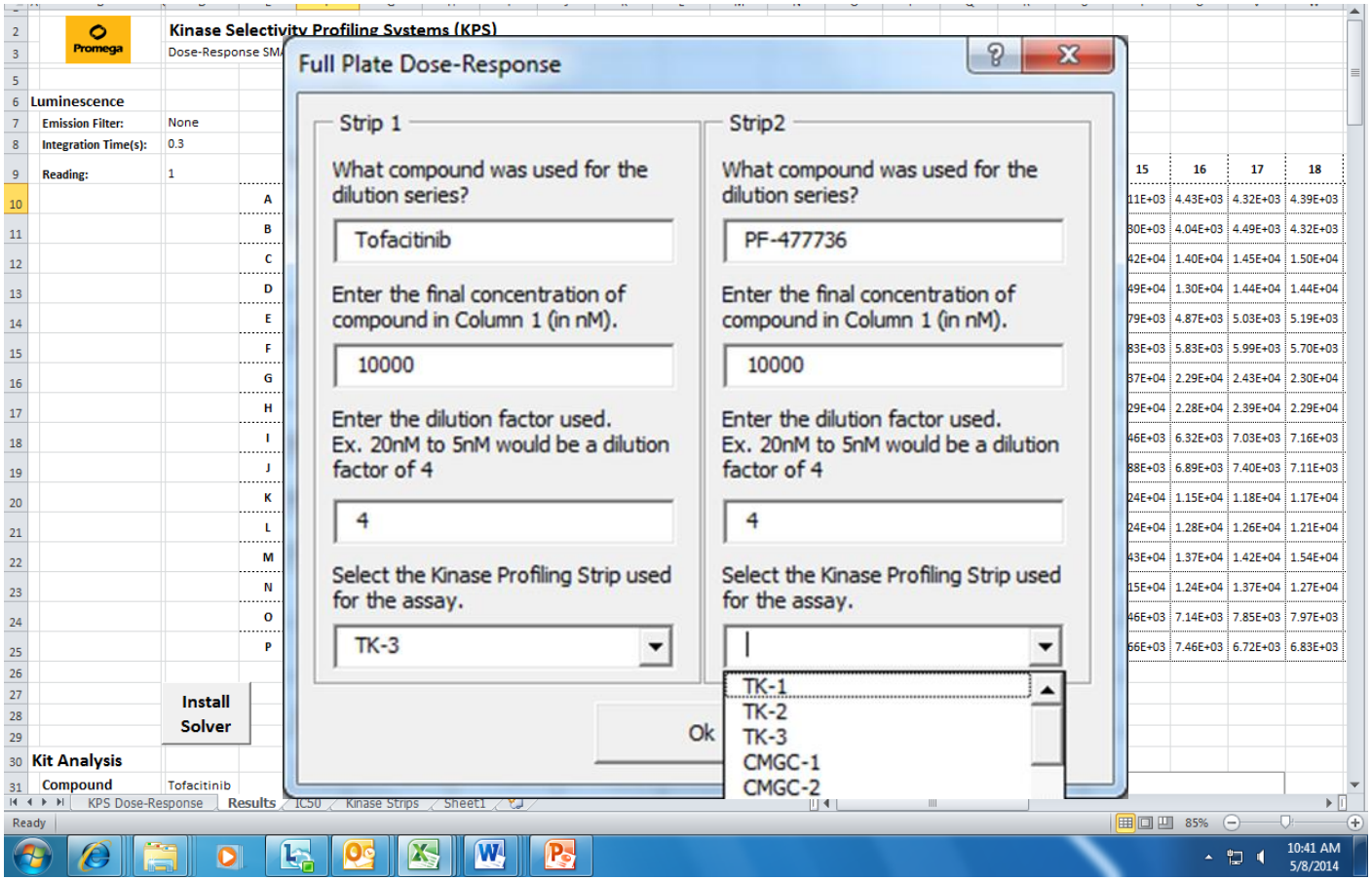
Data generated in a format that can be even published



Dose Response SMART Protocol

Raw data is imported into the template. Click to analyze.

Answer simple questions about the experiment: Strip kind, inhibitors concentration…



Dose Response SMART Protocol

Data generated in a format that can be even published with accurate IC50 determination

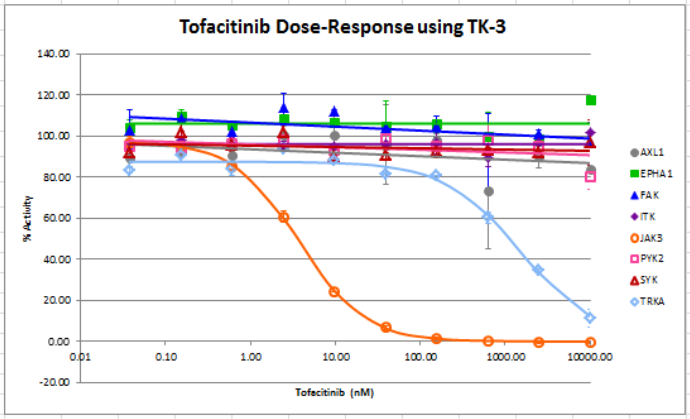
Install Solver

Click To Analyze

Kit Analysis

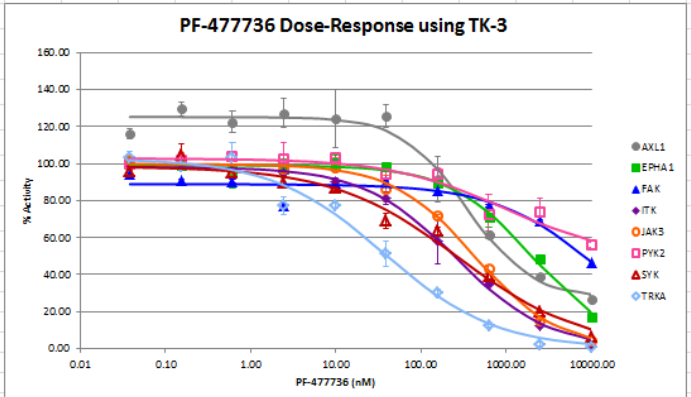
Compound	Tofacitinib
Starting Conc. (nM)	10000
Dilution Factor	4
KPS	TK-3

	IC50
AXL1	N/C
EPHA1	N/C
FAK	N/C
ITK	N/C
JAK3	4
PYK2	N/C
SYK	N/C
TRKA	1519



Compound	477736
Starting Conc. (nM)	10000
Dilution Factor	4
KPS	TK-3

	IC50
AXL1	341
EPHA1	2042
FAK	N/C
ITK	258
JAK3	429
PYK2	966
SYK	275
TRKA	37



KPS Dose-Response | Results | IC50 | Kinase Strips | Sheet1

Ready

Flexible profiling: One Dose or Dose response

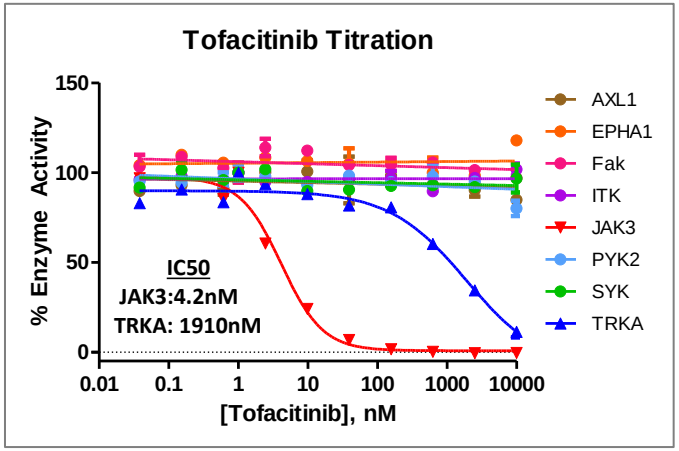
Single dose Profiling
(Up to 8/strip)

	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

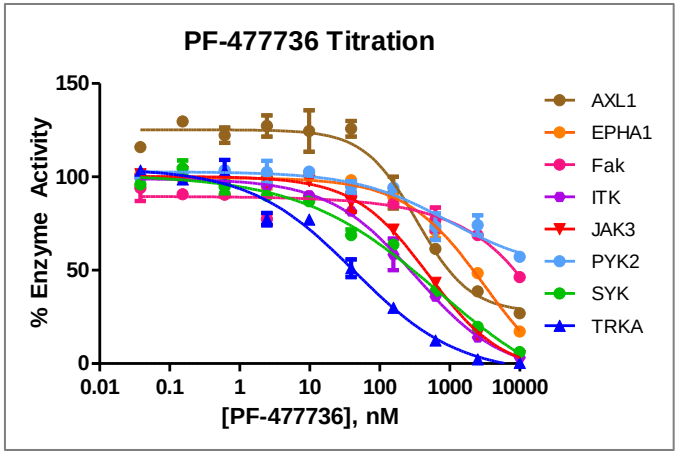
Inhibitor Concentration = 1 µM

Dose response Profiling
(Up to 2/strip)

Selectivity



Promiscuity

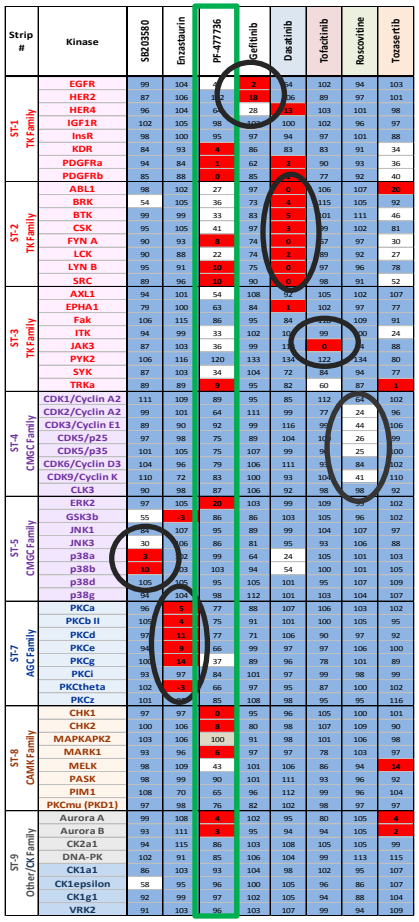


- Enabling flexible Kinase Profiling with the Strip Systems.
- Verified selectivity profiles against 8 kinases at once.

Large selectivity panels created with the profiling strips

64 Kinases with 8 compounds at a single dose profiling

ADP-Glo profiling systems



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

- **Selective and promiscuous compounds are identified.**
- **Inhibitor potency compared to different members of the same family or different families in the kinome.**

Inhibitors tested at 1µM

Inhibitors	Gefitinib	Dasatinib	Tofacitinib	SB203580	Roscovitine	PF-477736	Tozasertib	Enzastaurin
Kinase	EGFR	Abl/Src family	JAK	p38	CDK	CHK	Aurora	PKC

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

Validation of data created with ADP-Glo Profiling Systems

Comparison of ADP-Glo™ profiling to published radiometric data

ADP-Glo™ profiling systems

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

Radiometric data[1]

Dasatinib	Tofacitinib	Roscovitine	Tozasertib
64	102	94	103
106	89	97	101
13	103	101	98
100	102	96	97
94	97	101	88
83	83	91	34
2	90	93	36
1	77	92	40
0	106	107	20
4	115	105	92
5	101	111	46
3	99	102	81
0	67	97	30
2	89	92	27
0	97	96	78
0	98	91	52
92	105	102	107
1	102	97	77
84	118	109	91
101	99	100	24
113	0	94	88
134	122	134	80
72	84	94	77
82	60	87	1
85	112	64	102
99	77	24	96
116	99	44	106
104	100	26	99
99	96	25	100
111	93	84	102
93	104	41	110
92	98	98	92
99	109	99	102
103	105	96	102
99	104	107	97
95	93	106	88
24	105	101	103
54	100	101	105
101	95	107	109
101	103	104	107
107	106	103	102
101	100	105	95
106	90	97	92
97	97	106	100
96	78	101	89
97	99	98	99
95	87	100	102
98	95	95	116
96	105	100	101
98	107	109	90
98	101	106	98
97	78	103	97
106	86	94	14
111	93	96	92
112	99	96	104
102	98	97	97
95	80	105	4
94	94	105	2
108	105	105	99
104	99	113	115
98	102	95	107
105	96	86	107
105	94	88	104
107	99	94	109

Dasatinib	Tofacitinib	Roscovitine	Tozasertib	Kinase
21	96	97	78	EGFR
80	109	103	102	HER2
4	100	98	93	HER4
97	101	98	44	IGF1R
97	97	94	52	InsR
103	106	97	85	KDR
2	104	98	71	PDGFRa
2	95	91	72	PDGFRb
3	101	102	14	ABL1
5	101	103	95	BRK
2	99	83	99	BTK
7	101	89	78	CSK
2	94	106	72	FYN A
0	80	81	12	LCK
2	99	105	63	LYN B
4	100	107	27	SRC
61	98	100	44	AXL1
3	98	97	78	EPHA1
103	103	96	100	Fak
102	99	105	99	ITK
93	2	97	92	JAK3
98	100	100	95	PYK2
65	107	103	99	SYK
97	94	98	7	TRKa
96	96	83	99	CDK1/Cyclin A2
92	86	24	88	CDK2/Cyclin A2
92	92	58	95	CDK3/Cyclin E1
102	100	31	94	CDK5/p25
100	99	31	100	CDK5/p35
103	111	96	121	CDK6/Cyclin D3
84	100	79	93	CDK9/Cyclin K
95	106	105	110	CLK3
93	89	88	91	ERK2
97	100	96	104	GSK3b
106	108	98	95	JNK1
100	100	101	107	JNK3
37	93	96	97	p38a
77	103	101	93	p38b
99	94	97	90	p38d
100	97	95	96	p38g
103	79	87	82	PKCa
89	88	96	90	PKCb II
100	75	102	98	PKCd
102	99	98	98	PKCe
86	91	96	94	PKCg
101	103	92	99	PKCi
97	89	90	86	PKCtheta
101	92	100	76	PKCz
96	94	94	77	CHK1
103	104	97	100	CHK2
100	97	101	101	MAPKAPK2
107	119	115	114	MARK1
81	79	85	53	MELK
110	105	93	99	PASK
102	102	105	99	PIM1
99	97	99	102	PKCmu (PKD1)
93	84	89	5	Aurora A
93	99	95	5	Aurora B
105	104	106	114	CK2a1
N/A	N/A	N/A	N/A	DNA-PK
98	100	91	97	CK1a1
90	101	81	99	CK1epsilon
97	101	94	99	CK1g1
98	100	107	107	VRK2

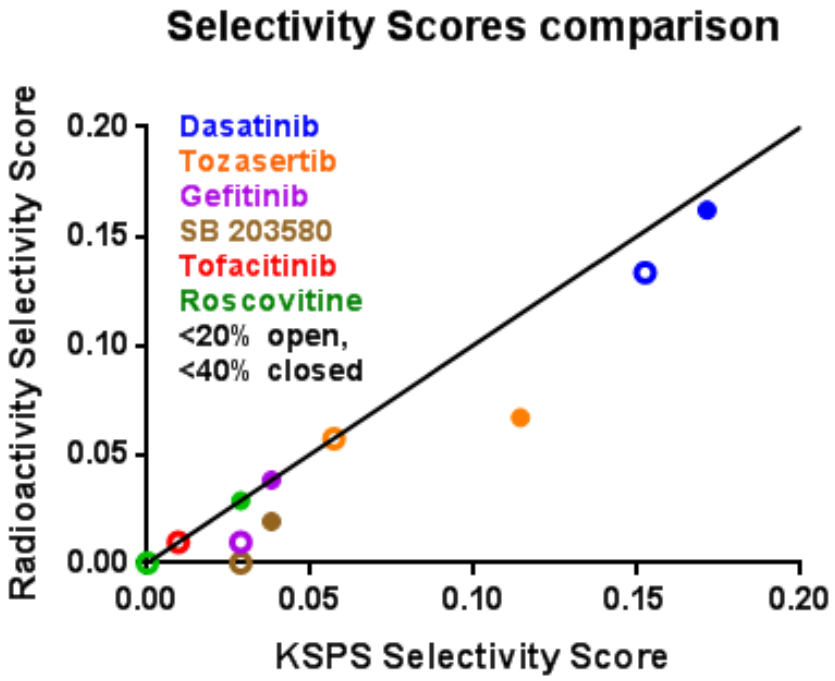
Data generated with ADP-Glo™ platform is consistent with published potencies of radioactivity-based Kinome profiling.

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

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

Validation of data created with ADP-Glo Profiling Systems

Comparison of Selectivity profiling Scores* between ADP-Glo™ and radiometric data (Over 100 kinases compared)

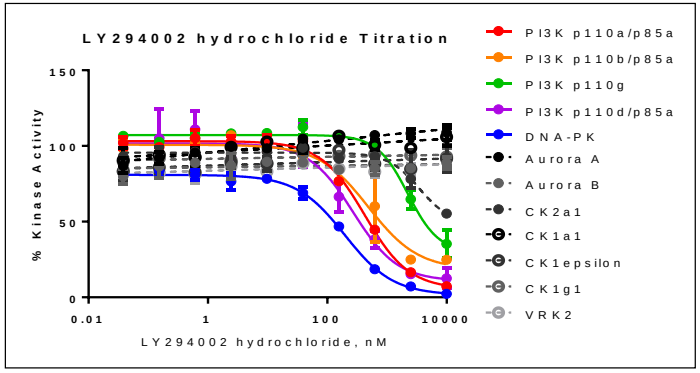


Selectivity Data generated with ADP-Glo™ platform correlates perfectly with radioactivity-based selectivity data.

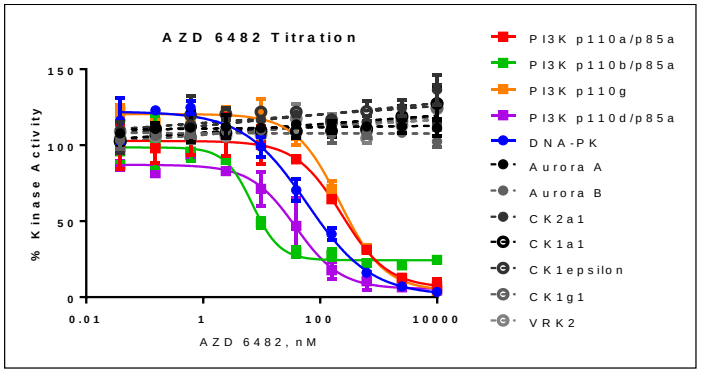
***Selectivity scores: # of kinases with % activity left < 20 or 40% / # of kinases tested**

Simultaneous profiling compounds against protein and lipid kinases.

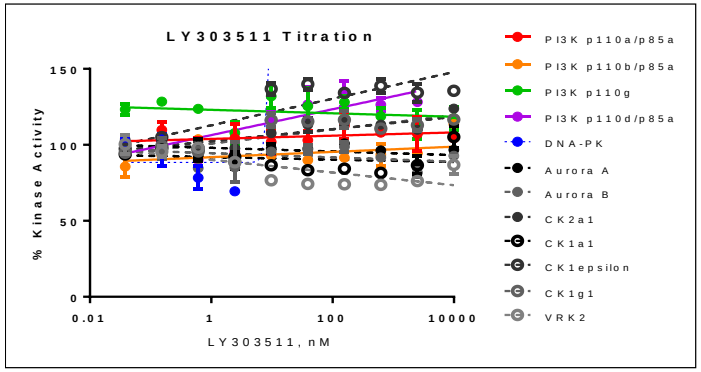
Profiling PI3K family inhibitor



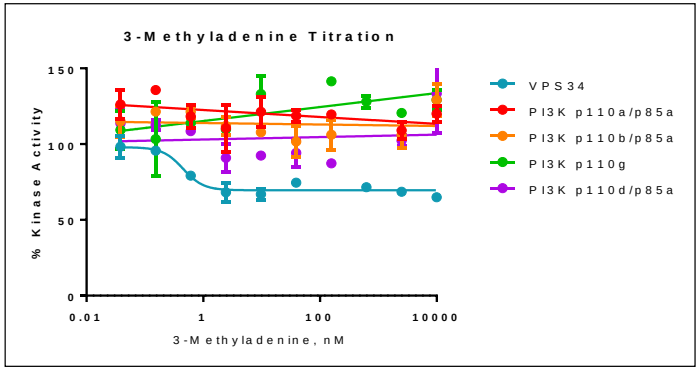
Profiling PI3Kβ inhibitor



Testing control compound inactivity



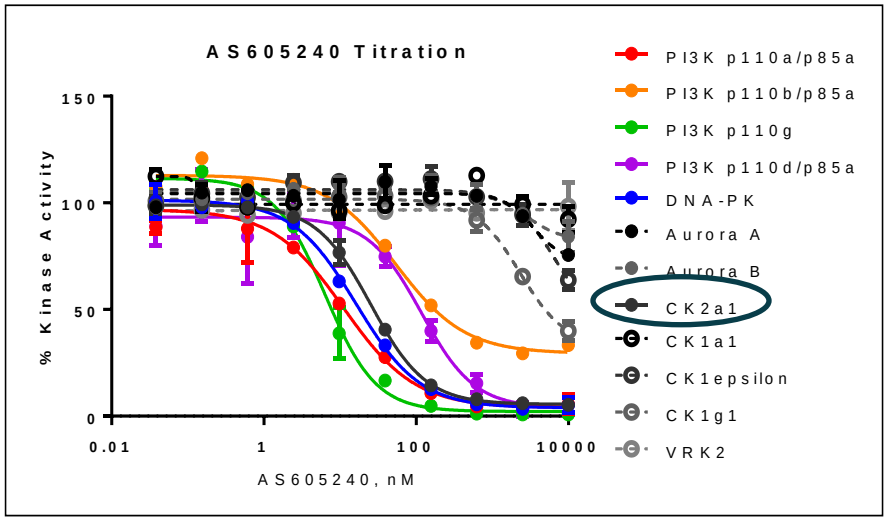
Profiling Lipid Kinase Class III inhibitor



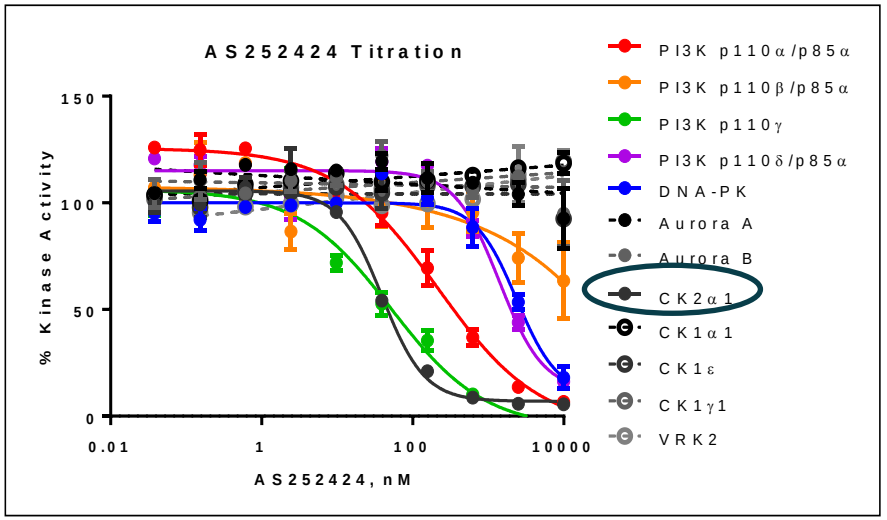
Profiling kinases in strips to identify compounds' selectivity towards members of PI3 Kinase family.

Simultaneous profiling compounds against protein and lipid kinases.

Profiling PI3Kγ inhibitor



Profiling PI3Kγ inhibitor



Simultaneous Profiling of Protein and Lipid kinases identifies promiscuity of PI3K specific inhibitor towards other protein kinases such as CK2α1.

Advantages of Kinase profiling strips

Kinase Selectivity Profiling Strip Systems have the following advantages:

- ✓ **Fast and simple reaction assembly:** Two quick dilutions provide working stocks of kinases and substrate/co-factor solutions sufficient for 25 kinase reactions.
- ✓ **One-time use design**: Eliminating multiple freeze/thaw cycles ensures optimal kinase activity per experiment.
- ✓ **Optimized kinase activity for inhibitor profiling:** All kinases have been optimized to provide optimal ADP production when assayed at 10µM ATP.
- ✓ **Formatted strips provide access to eight kinases at a time:** Kinase from singular kinase families are grouped together for a 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 the 8 kinases at once.

Advantages of Kinase profiling strips

ADP-Glo™ Kinase Selectivity Profiling Systems generate data similar to the "gold standard" Radiometric assay

"Now even chemists can do kinase profiling?"

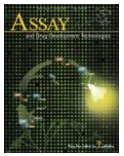
General features of ADP-Glo™ Kinase platform

ADP-Glo™ platform offers so many positive attributes that make it ideal for all stages of drug discovery

Basic Research, Primary and secondary screenings and for profiling of lead compounds.

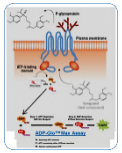
Summary

ADP-Glo™ Kinase Platform



1. ADP-Glo Kinase Assay

Kinase reactions up to 1mM ATP



4. ADP-Glo Max Assay

ATPase reactions up to 5mM ATP (ABC transporters,…)

2. Kinase Enzyme Systems

174 complete kinase assays

3. Kinase profiling Systems

8 well kinase strips for easy profiling

Streamlining Kinase Profiling Workflow by Integrating Bioluminescent Kinase Strips with Multimode Detection System

Hicham Zegzouti, Ph.D. **Assay Design Group, Promega, Madison WI**

SLAS 2015 Tutorial

February 10, 2015

Promega Corporation

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