Promega Corporation

ADP Detection Platform for kinase inhibitor screening, mode of action studies and profiling

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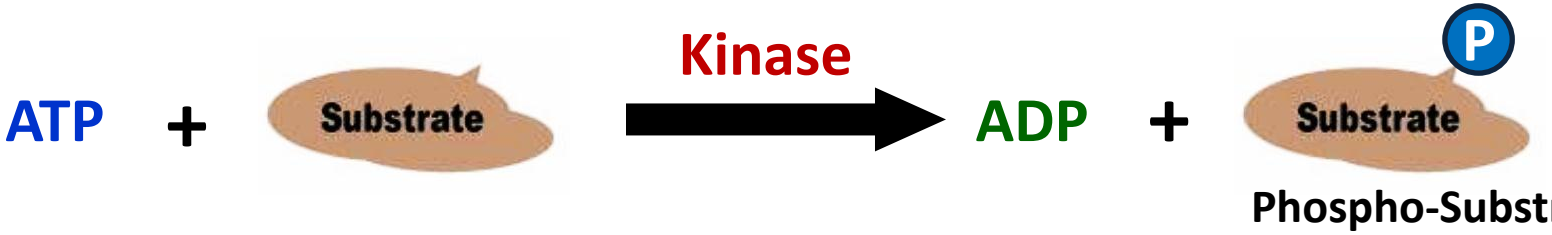
Kinases orchestrate complex biological processes

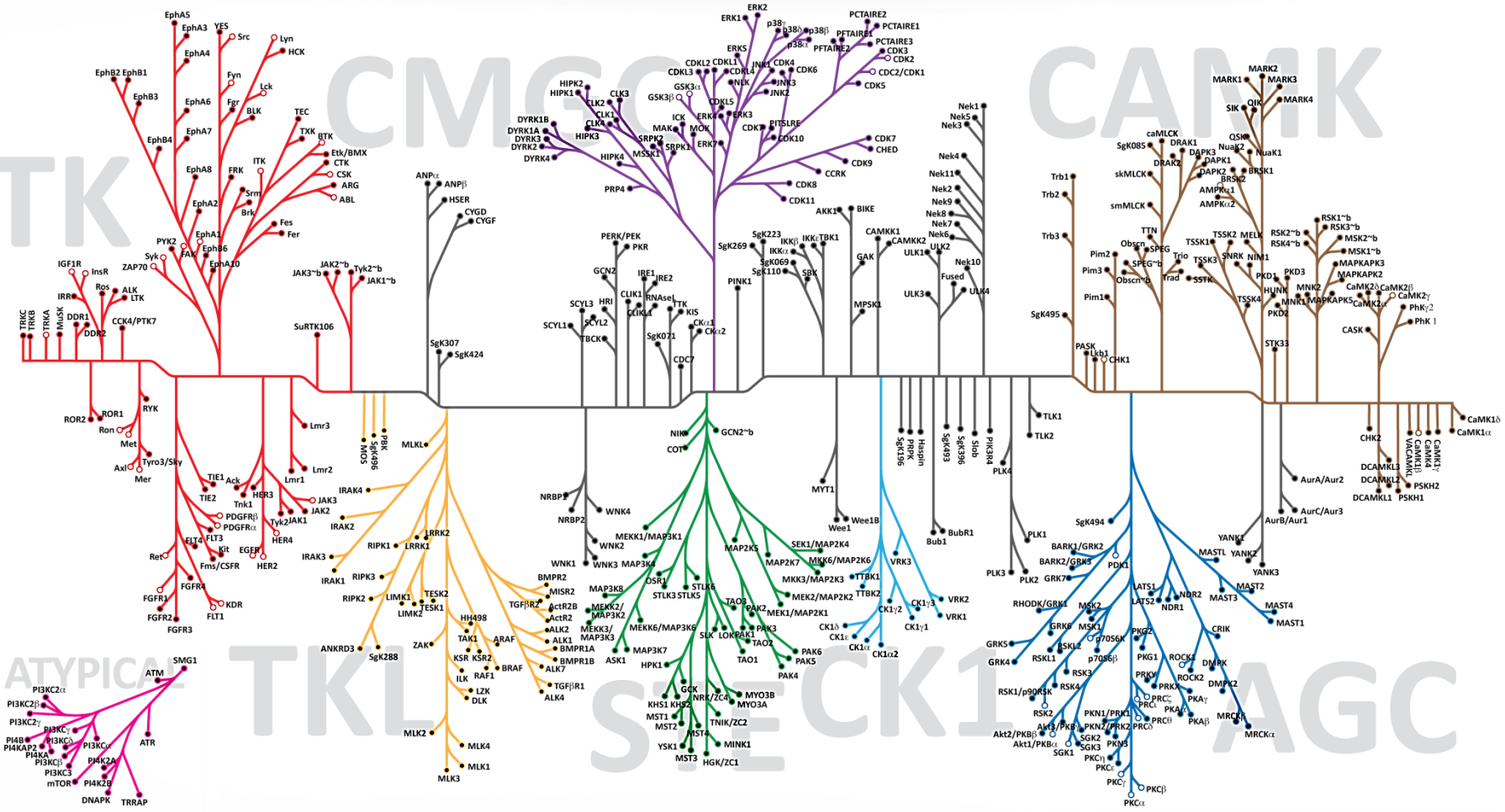
Kinases play a critical role in Human biology

- **Important components of Cell Signal transduction**
- **Regulation of many cellular processes through phosphorylation of diverse substrates (proteins (S/T, Y), Lipids, Sugars…)**



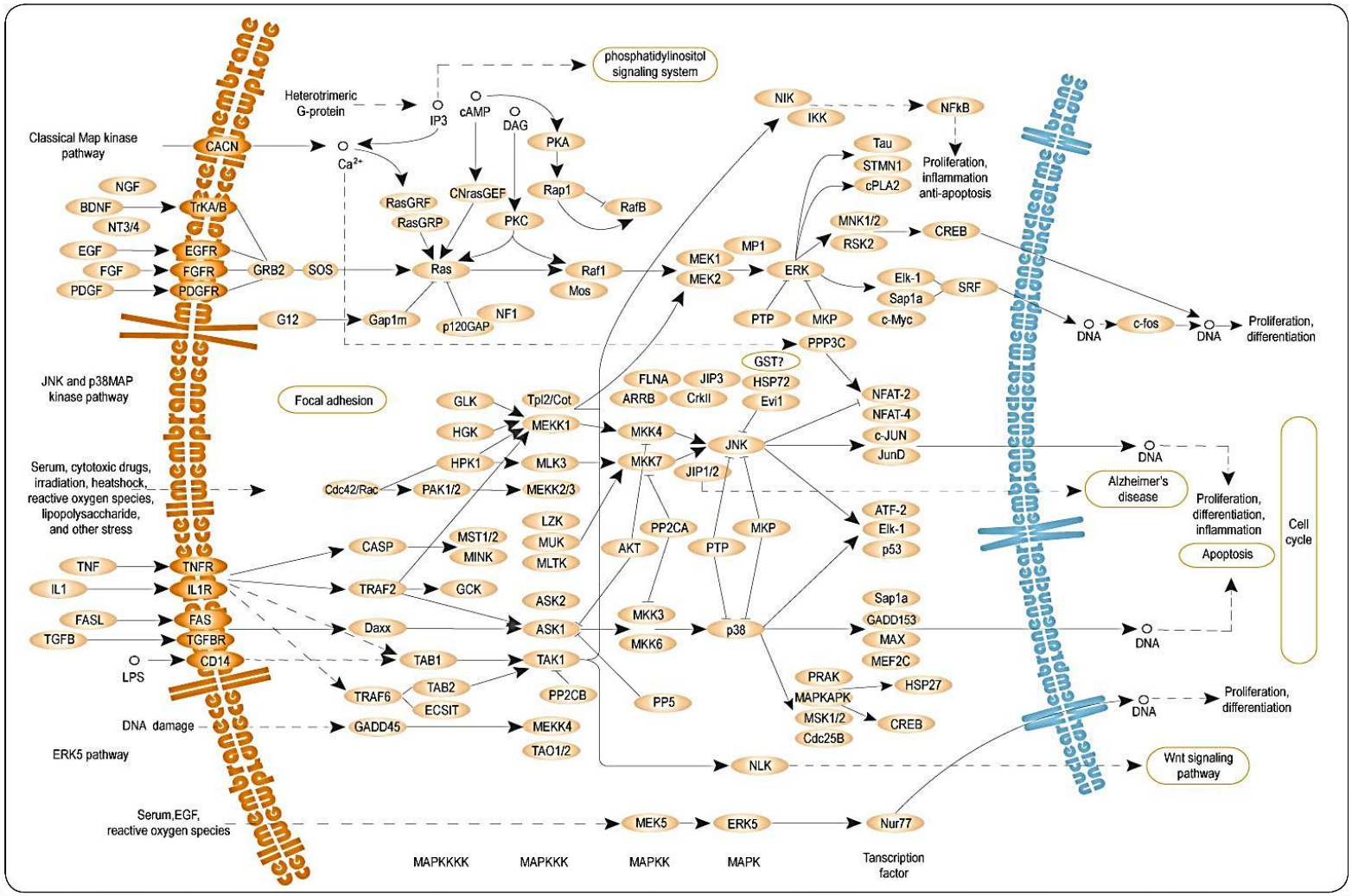
- **~518 protein Kinase in human genome (388 S/T, 90 Tyr, 40 atypical) & more**
- **More than third of all human proteins are phosphorylated**

Human Kinome



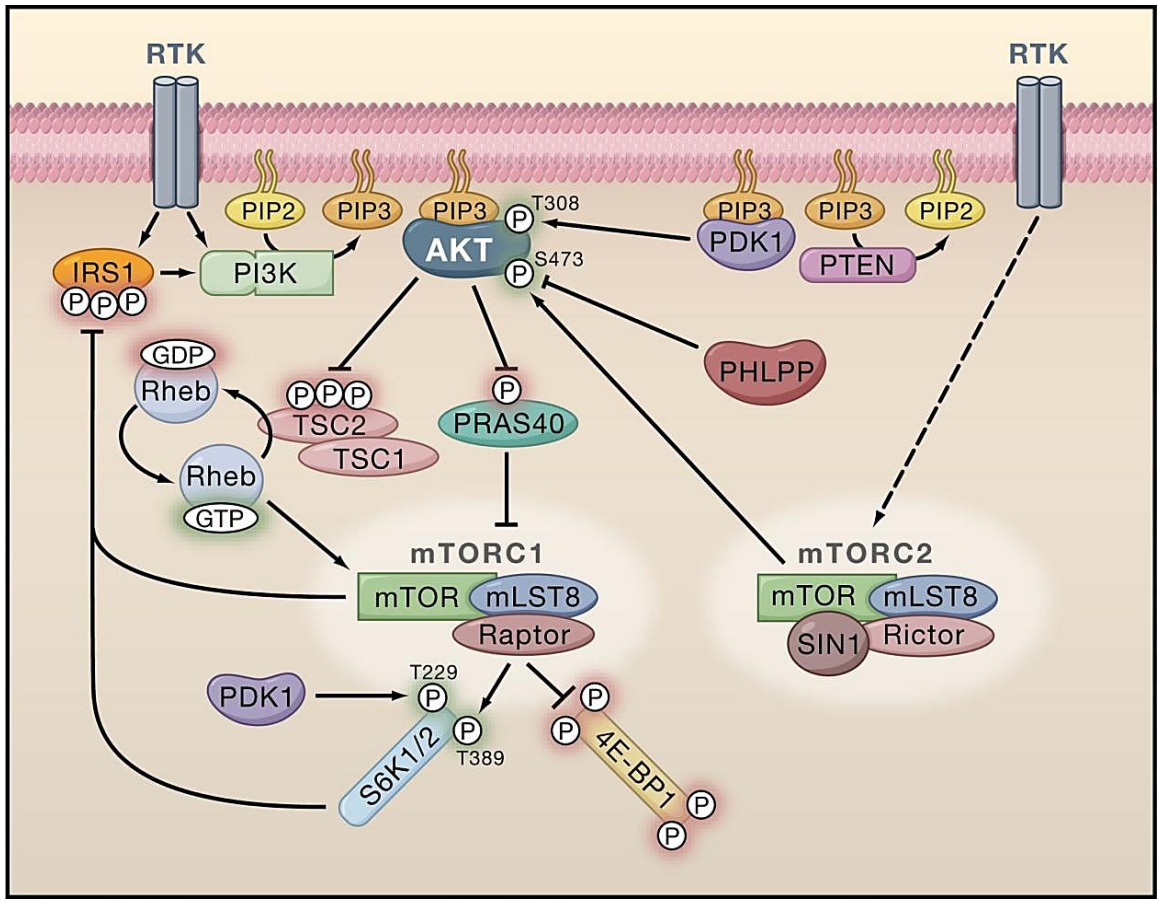
MAP Kinase pathway example

Signal Transduction (Phosphorylation cascades) from the receptors to the transcription factors



Akt-Lipid Kinase Signaling example

Cross talk between Signal Transduction pathways



Regulation of:
- **cell survival**
- **Proliferation**
- **insulin-dependent metabolic responses.**

Manning and Cantley (2007) *Cell* **129**, 1261-1274.

Kinases orchestrate complex biological processes

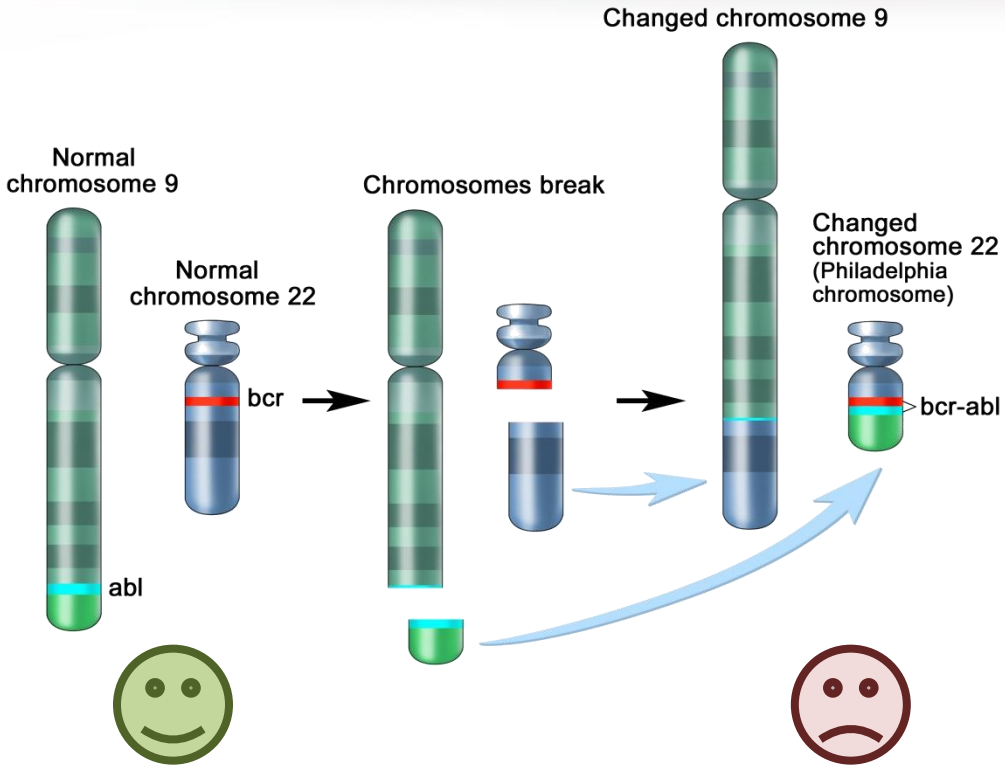
Kinases play a critical role in Human biology

Kinases are directly involved in many diseases

- **Under pathological conditions Kinases can be deregulated/mutated**
- **Alteration in phosphorylation states results in abnormalities**
- **Over 400 Human Diseases are Linked to Defects in Kinases- (and Ppases) Dependent Signaling Pathways**

Kinases became the largest target in the drug discovery market

bcr-abl: An oncogene that started it all



abl

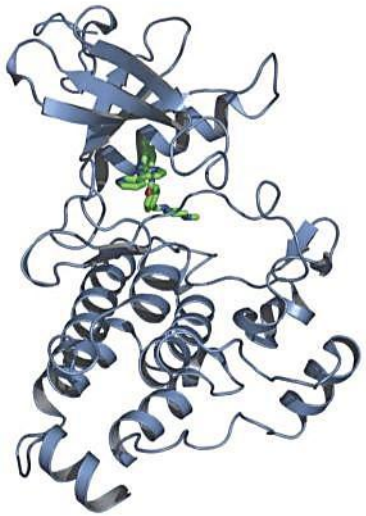
c-abl
Very **Low** Activity

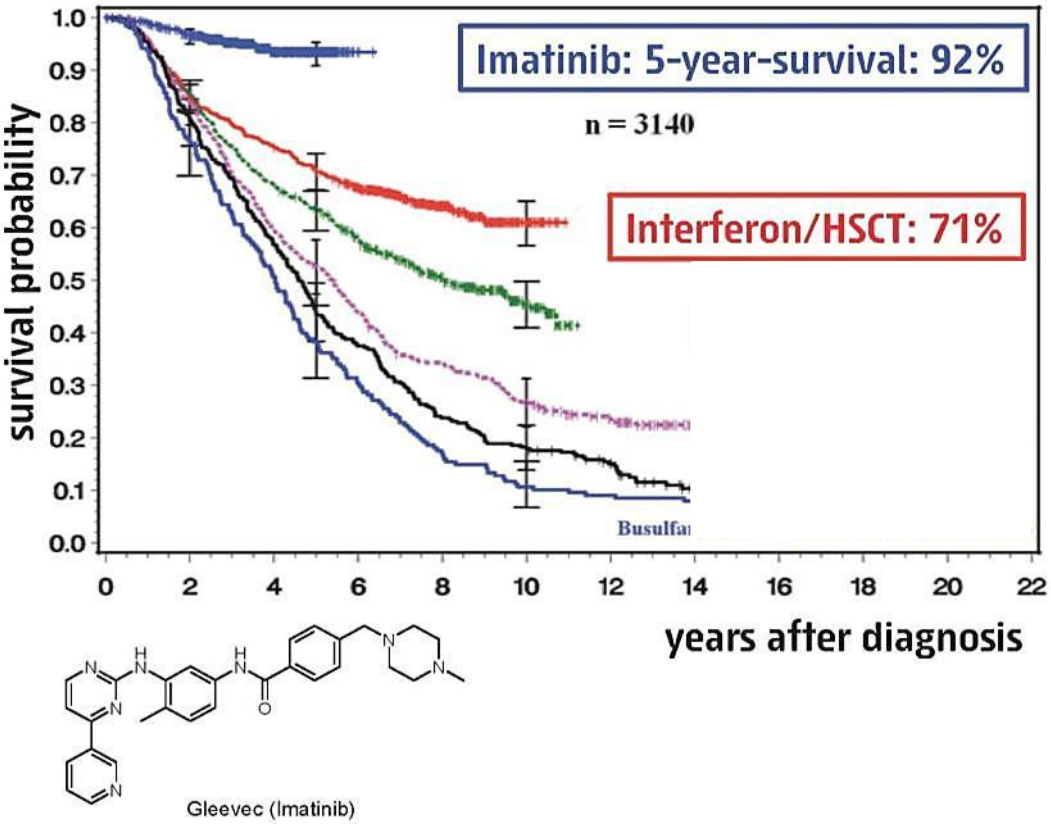
bcr | abl

bcr-abl
Very **High** Activity

- First chromosomal translocation identified
- Deregulated constitutively active Tyrosine Kinase
- Expressed in Chronic Myeloid Leukemia (CML)
- Expression sufficient to cause CML

Imatinib (Gleevec): Improved Long-term survival





Gleevec inhibits the DFG out state of the Kinase (Inactive form)

DFG (Asp-Phe-Gly): Mg-ATP binding site

Importance of Kinases in Drug discovery

US Marketed Small Molecule Kinase Inhibitors

- **Kinase**
- **Disease**
  Neurol

narket

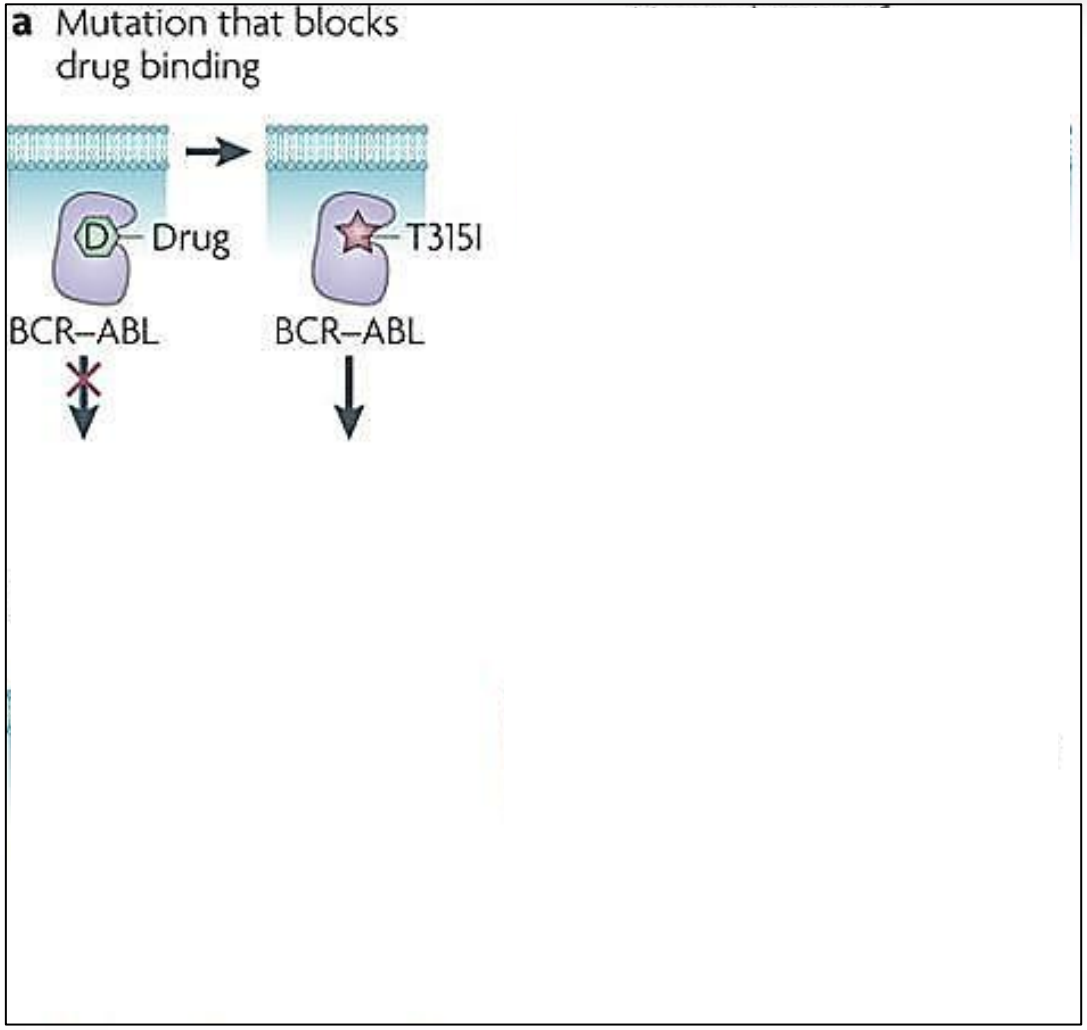
ascular,

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

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

Tumors become resistant to Kinase inhibitors



Resistance mechanisms:

1. **Mutation in kinase target**
2. **Induction of Bypass mechanism**
3. **Activation in up/down-stream effectors**

➢ **Patient relapse requires second-line therapies:**

- **Second generation of inhibitors needed**
- **Therapeutic combinations of multiple kinase targets**

Z. A. Knight, H. Lin & K. M. Shokat (2010) *Nature Reviews Cancer* **10**, 130-137

Combinatorial therapy as a new trend for recalcitrant cancer treatment

HIV treatment example:

Elvitegravir (integrase inhibitor)

Cobicistat (drug metabolism inhibitor)

Emtricitabine and Tenofovir (nucleoside RT inhibitors)

vs.

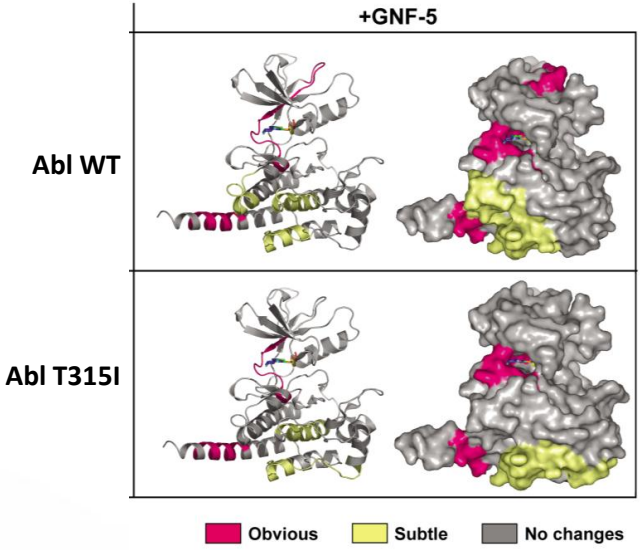
Cancer treatment example:

Kinase inhibitors are used as a single therapy

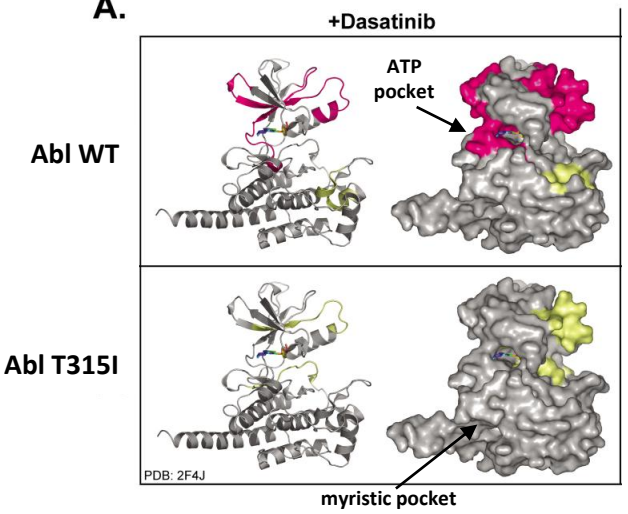
Combinatorial therapy as a new trend for recalcitrant cancer treatment (Abl example)

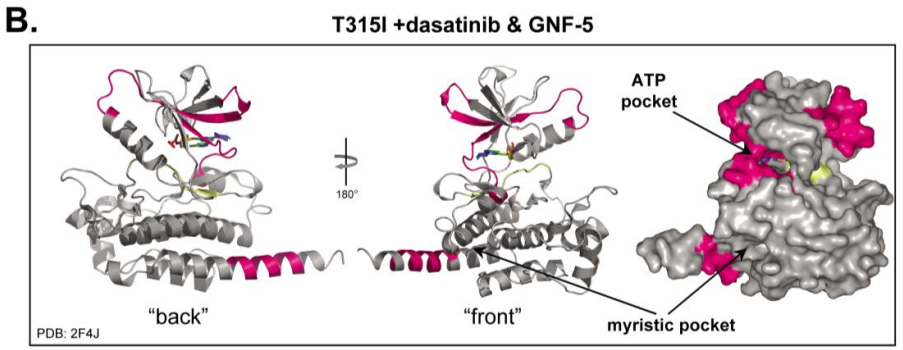
in combination with: Allosteric inhibitors binding to non ATP binding sites:

- PIF pocket
- Substrate pocket
- Myristic pocket



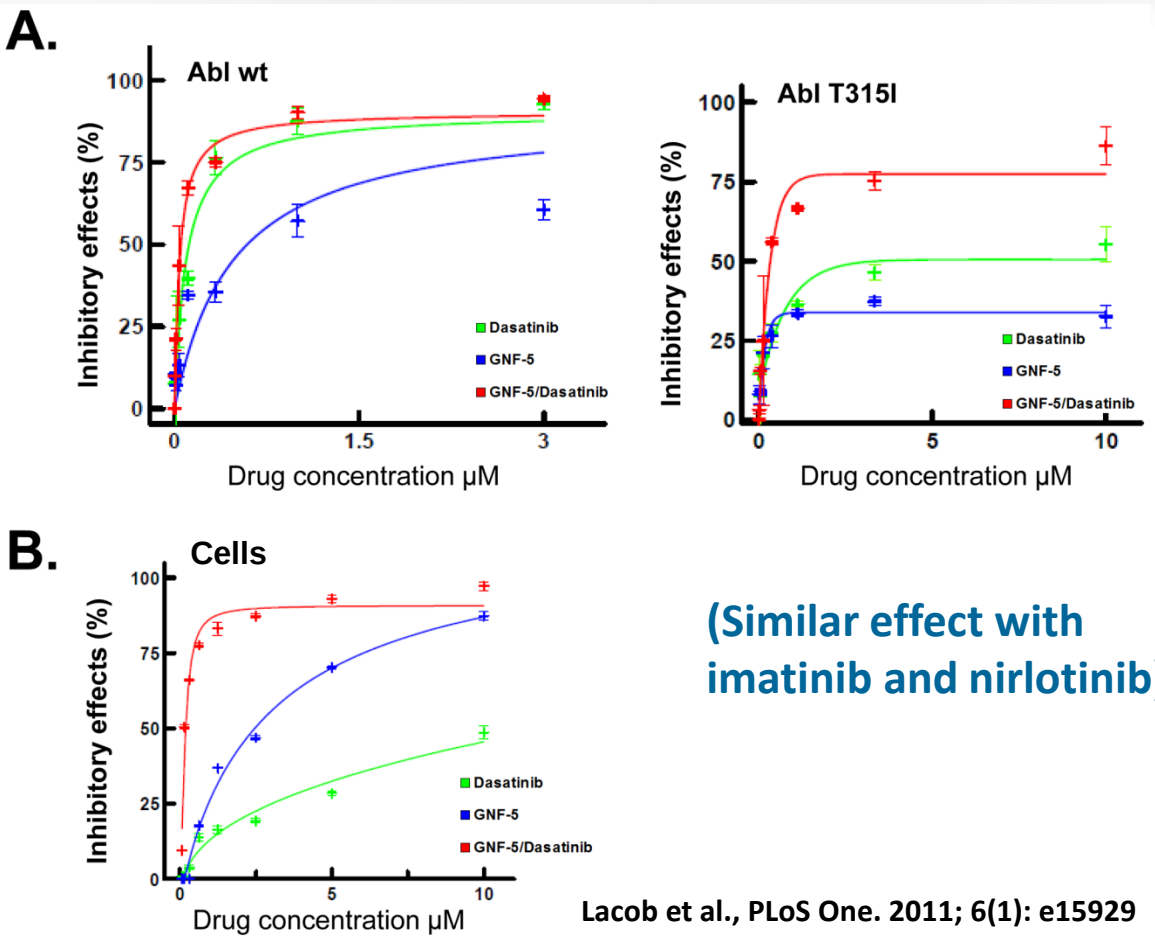
ATP site inhibitors





Simultaneous binding of Dasatinib and GNF-5 to T315I caused conformational changes in Abl such that dasatinib binding to T315I became similar as when it is bound to WT Abl.

Combinatorial therapy as a new trend for cancer treatment



(Similar effect with imatinib and nirlotinib)

Lacob et al., PLoS One. 2011; 6(1): e15929

Additive interaction between GNF-5 and the ATP-competitive inhibitor Dasatinib to inhibit proliferation of Bcr-Abl T315I mutant

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

Two way paradigm

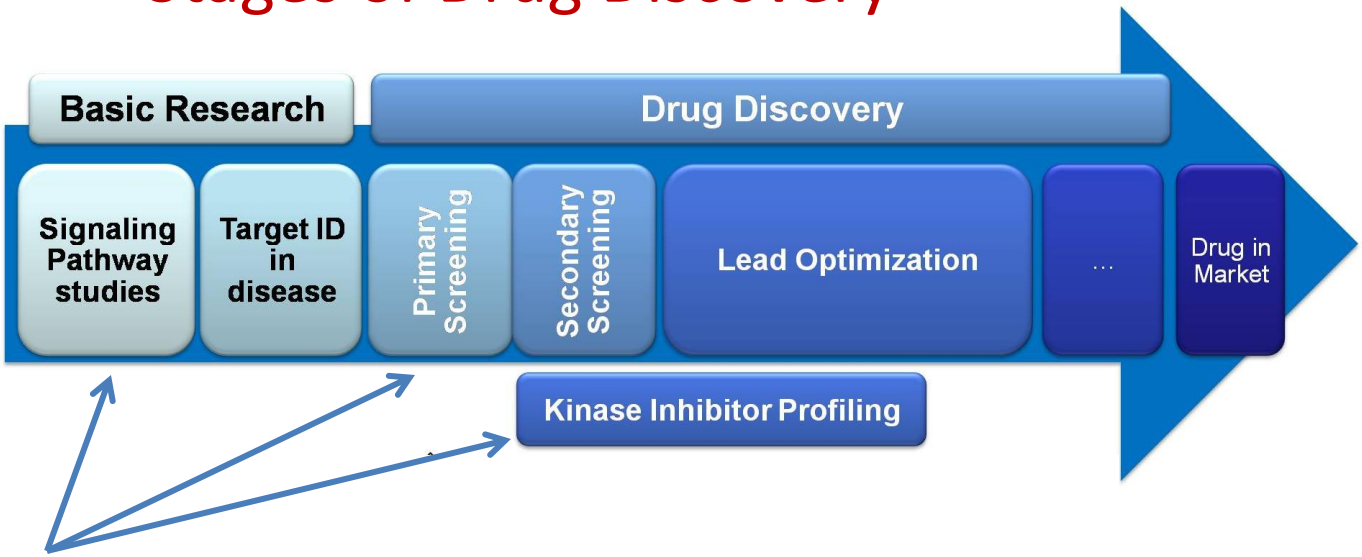
Selective compounds ⟷ Potency

Choosing the right assay for success

Kinase Studies in Basic Research and Drug Discovery

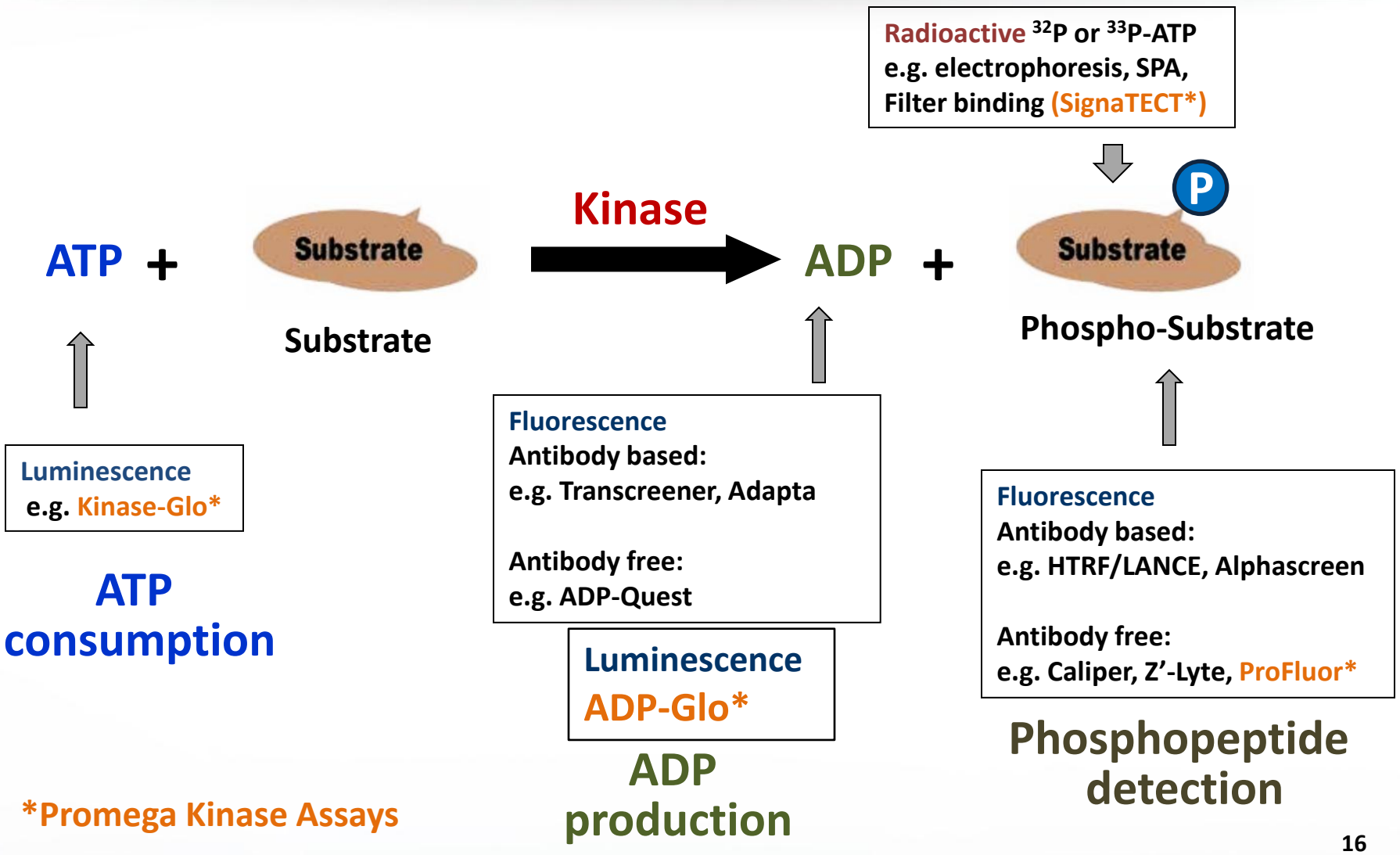
Biochemical Kinase Assays

Stages of Drug Discovery



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

Detection of Kinase activity



Current Kinase Assays features and drawbacks

Drawbacks of Current Assays:

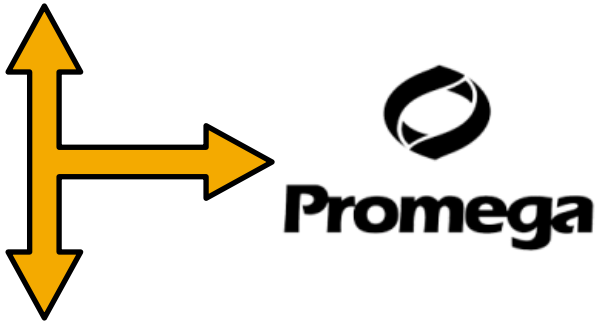
- If not radioactive they require specific antibodies
- Require fluorescently-labeled peptides
- False Hits with Fluorescence based assays
- Very expensive
- Require special detection technology
- Not tolerant to High concentrations of ATP

Features of an Ideal Assay:

- ✓ Homogeneous, Nonradioactive, Robust
- ✓ Universal (any enzyme substrate combinations)
- ✓ Applicable for different kinases and diverse substrates
- ✓ Use multiphosphorylated substrates
- ✓ Minimal False Hits
- ✓ Distinguish between ATP competitive and noncompetitive inhibitors

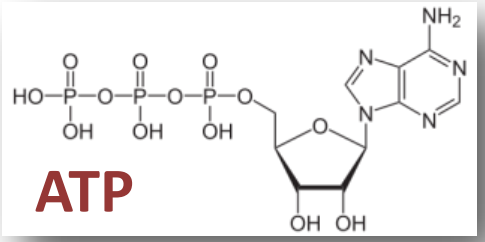
Kinase Research: parts of the equation

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



Pharmaceuticals

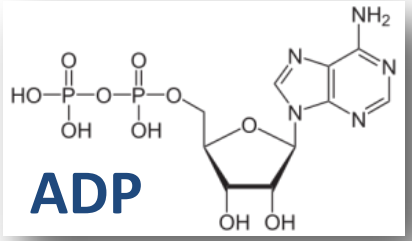
Bioluminescent Kinase Assay Platform



Kinase Glo® Assay

Monitoring ATP Depletion

- Kinase Glo® **(10µM ATP)**
- Kinase Glo® Plus **(100µM ATP)**
- Kinase Glo® Max **(500µM ATP)**

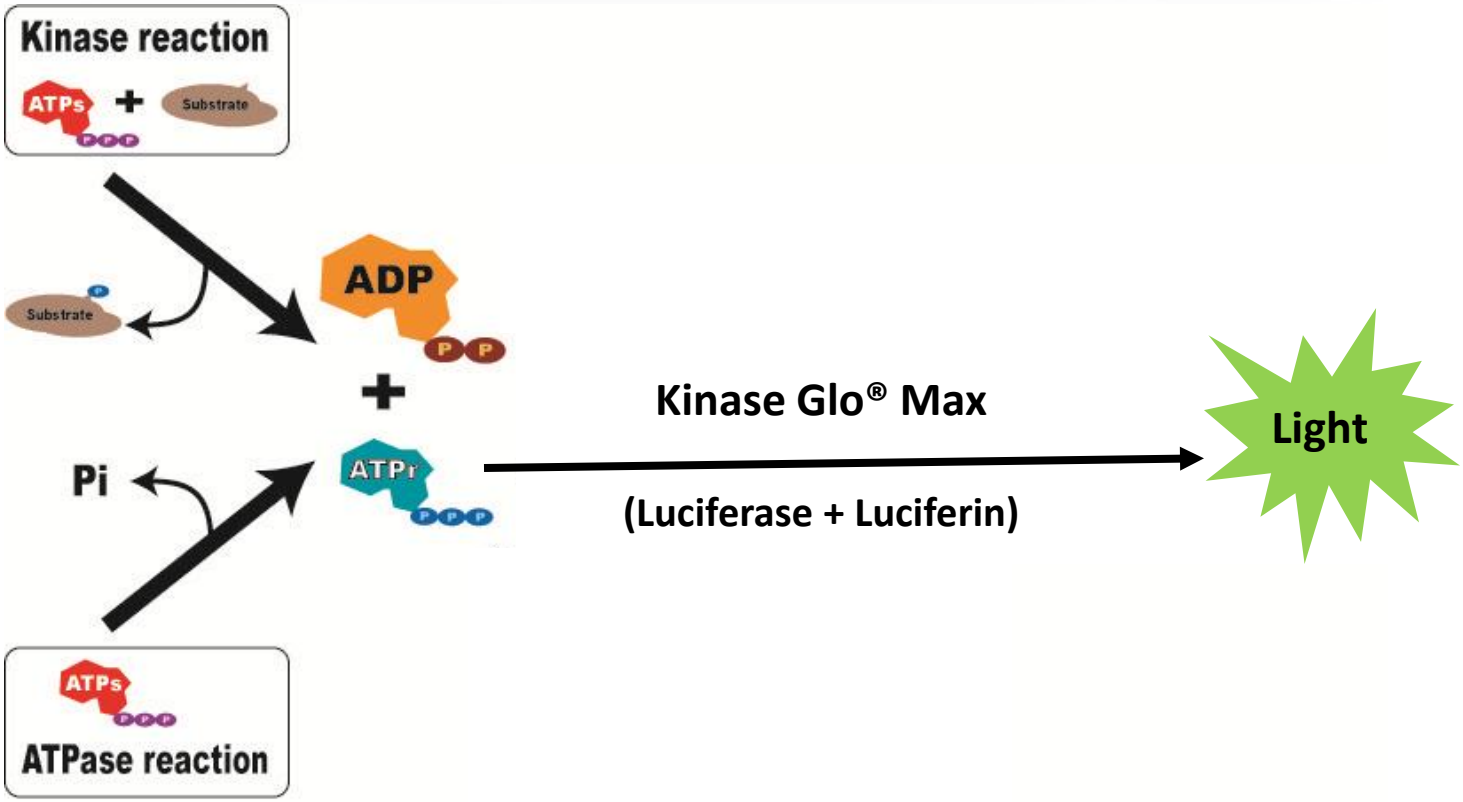


ADP Glo™ Assay

Monitoring ADP Production

Micro to Millimolar ATP Concentration

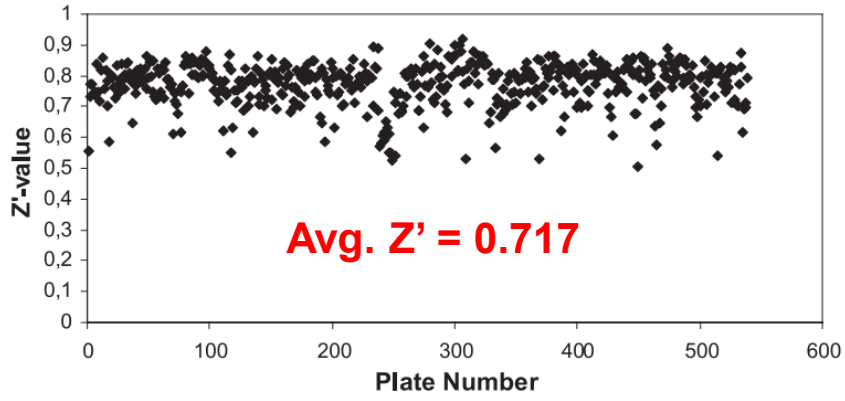
ATP detection: Kinase-Glo® Platform



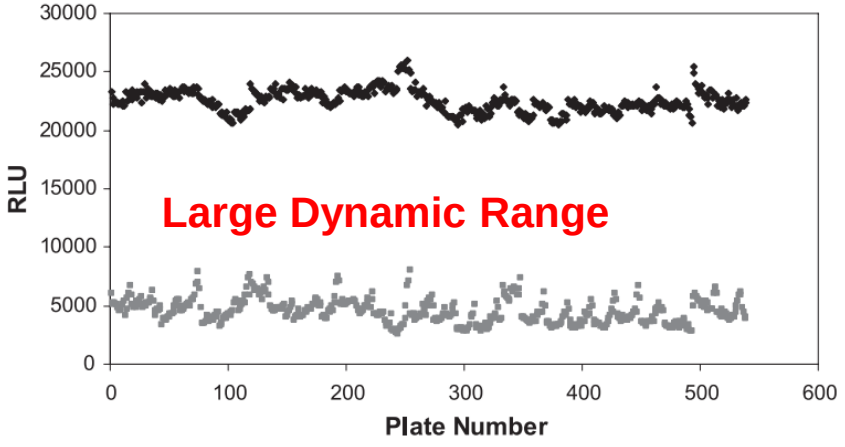
Light output is correlated with the amount of ATP remaining and is inversely correlated with the amount of kinase activity

Sensitivity and Robustness of Kinase-Glo®

Z' Values calculated for Test Plates



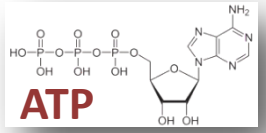
Averages for controls (+ and -)

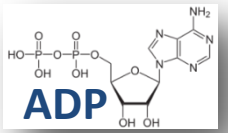


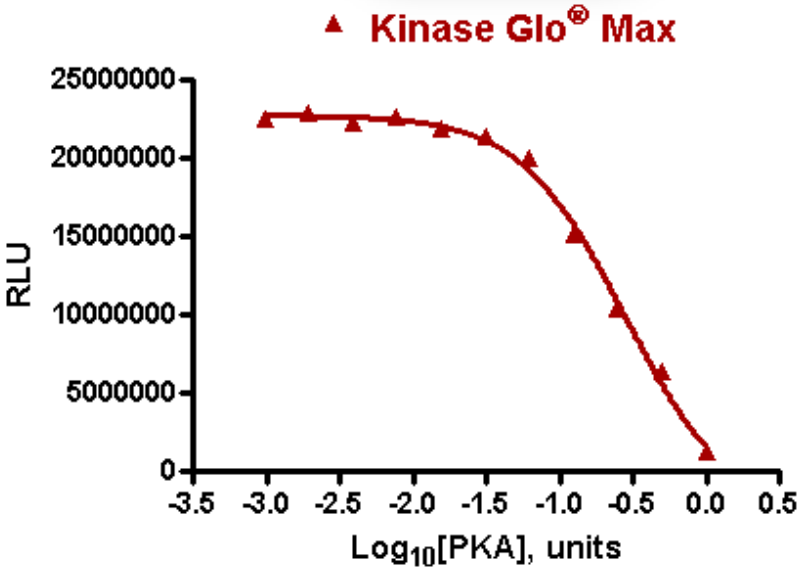
- ✓ **Kinase-Glo® Assay generates a high signal to background ratios**
- ✓ **Kinase-Glo® Assay is a robust assay ideal for HTS**

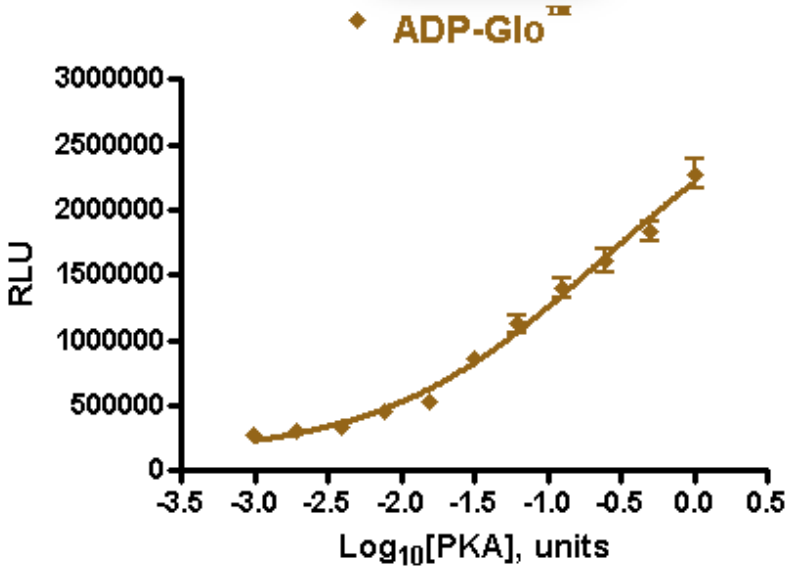
Baki A, *et a*l (2007)
Assay and Drug Dev. Technol. **5:** 75-83

Luminescent Kinase assays: Two Different Approaches



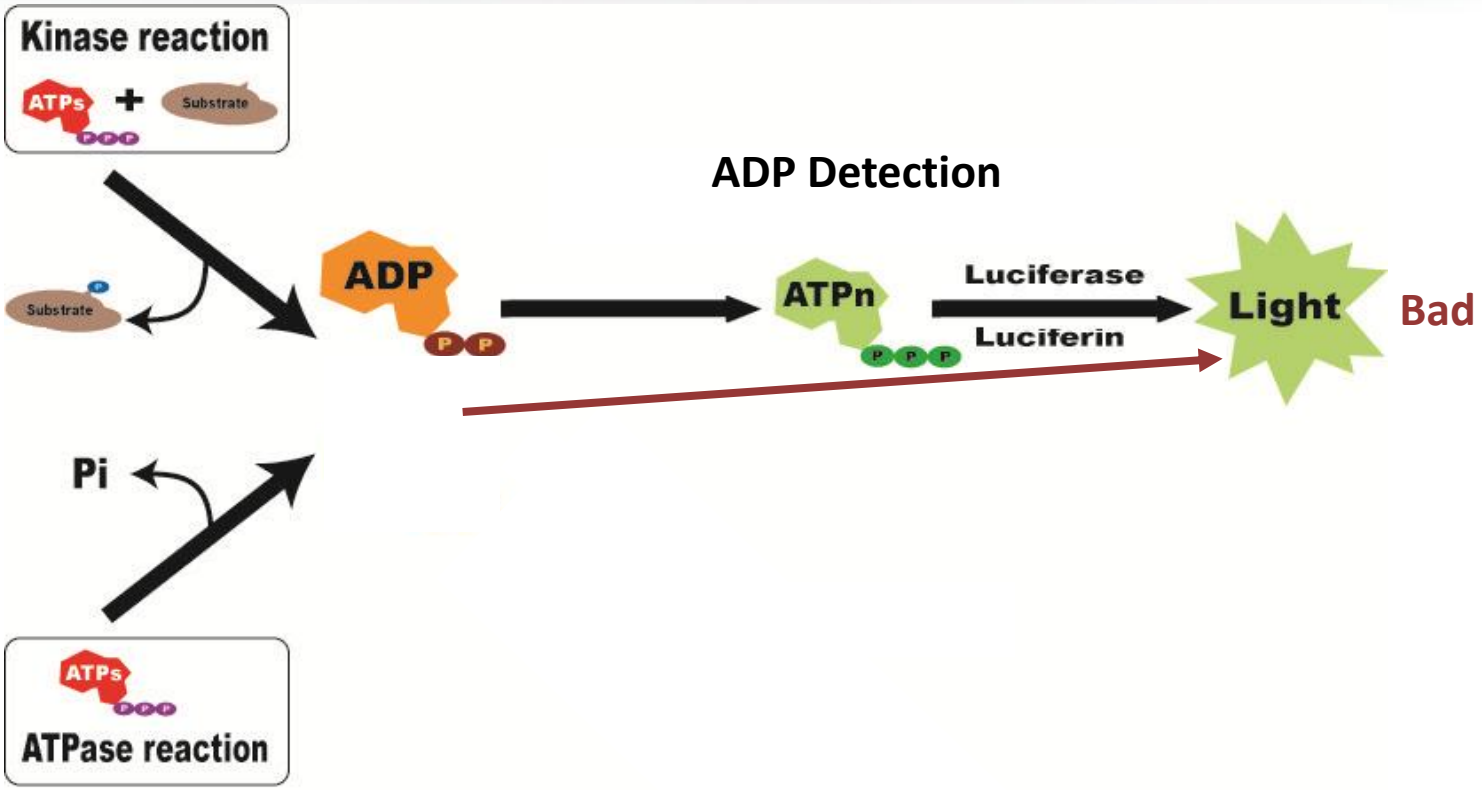






- Kinase-Glo® measures the remaining ATP after a kinase Reaction
- ADP-Glo™ measures ADP produced in a kinase Reaction

Luminescent ADP detection assay requirements



ADP-Glo™ Assay Format- 1:1:2

384-well plate

5µl kinase reaction

+

5µl ADP-Glo™ Reagent

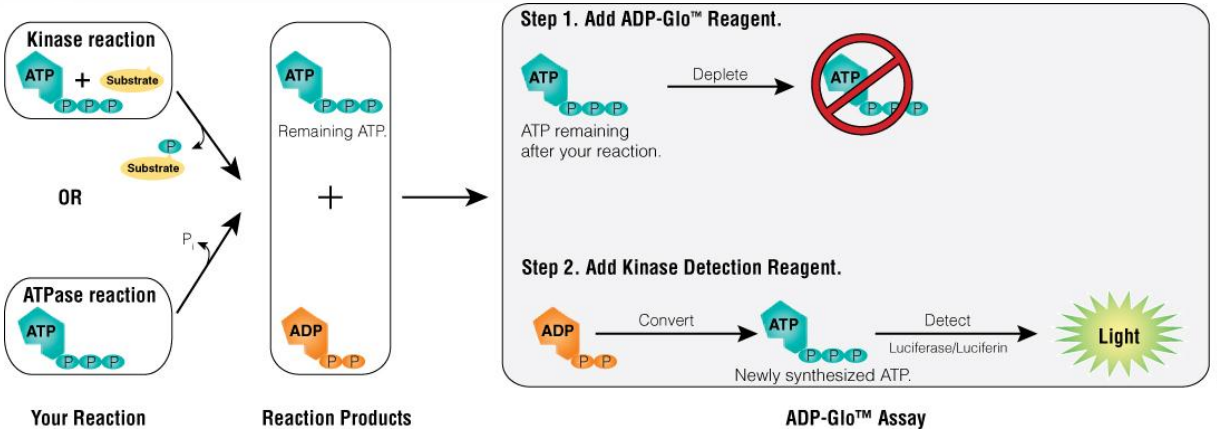
40 min. Incubation

+

10µl Kinase Detection Reagent

30-60 min. Incubation

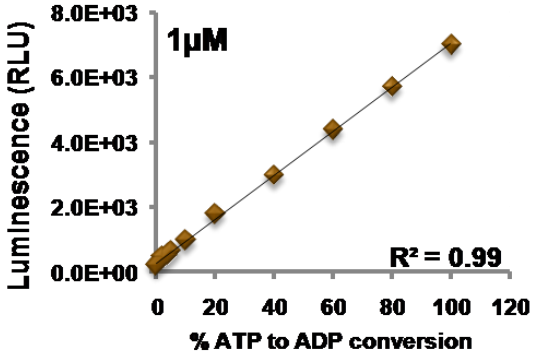
Record Luminescence

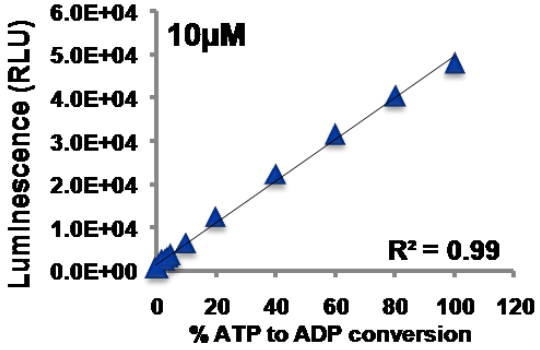


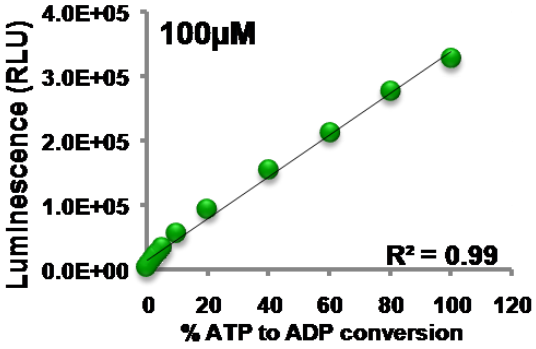
White Plates	Format 1:1:2 (µl)	
1,536-well	2.5/2.5/5	
384-well	5/5/10	10/10/20
96-well	25/25/50	50/50/100

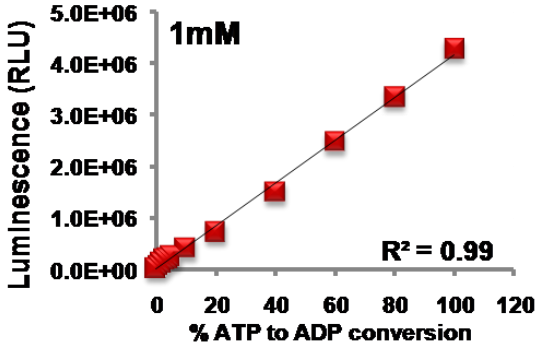
Linearity of the ADP-Glo™ assay

ADP conversion curves at different ADP/ATP concentrations









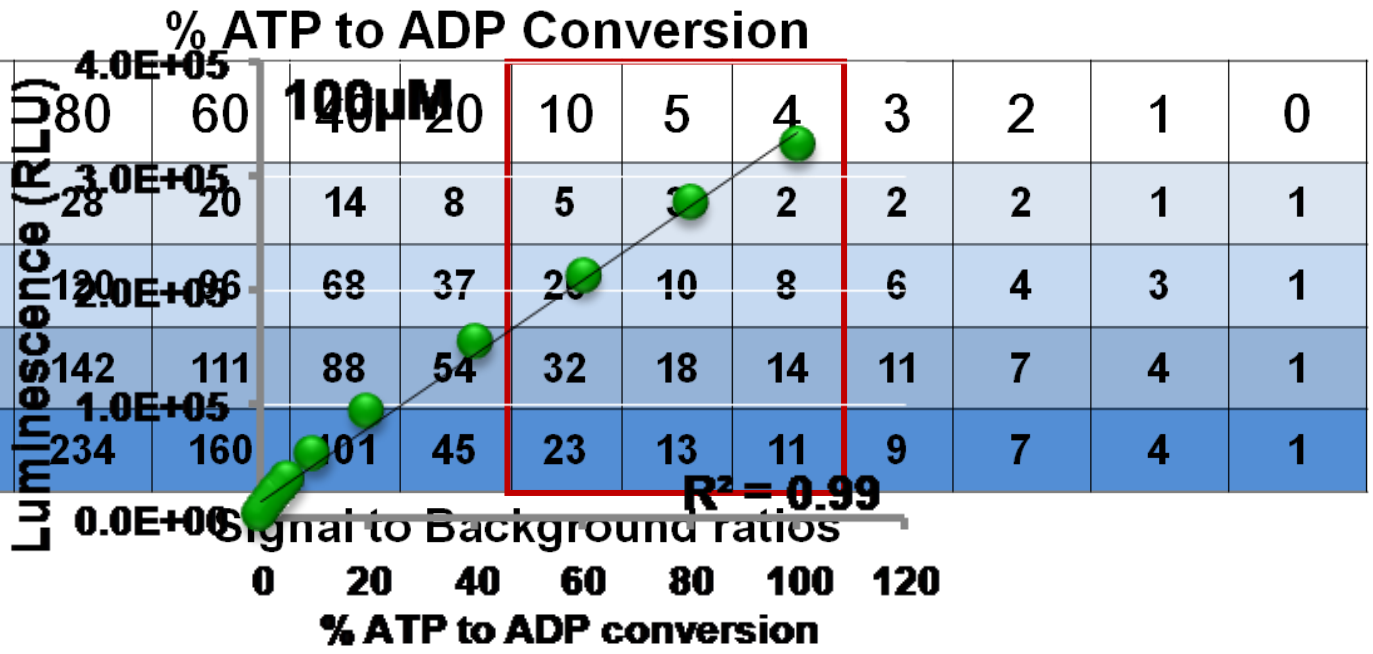
ADP-Glo™ can be used virtually with any ATP concentration

Sensitivity of the ADP-Glo™ assay

Promega

Signal to Background ratios produced at different ATP to ADP conversion %

ATP-ADP ranges	% ATP to ADP Conversion											
	100	80	60	40	20	10	5	4	3	2	1	0
1µM	34	28	20	14	8	5	3	2	2	2	1	1
10µM	145	120	86	68	37	20	10	8	6	4	3	1
100µM	167	142	111	88	54	32	18	14	11	7	4	1
1mM	309	234	160	101	45	23	13	11	9	7	4	1



ADP-Glo™ can detect as low as 20nM ADP in 5µl (0.1pmole) with a high Z' value

MORE Sensitivity of the ADP-Glo™ assay

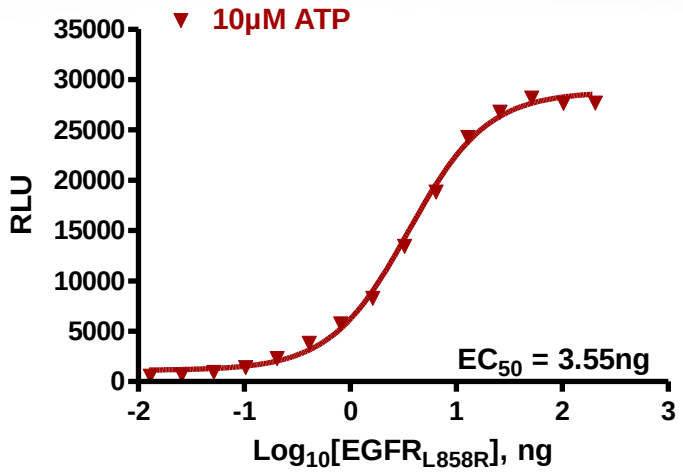
ADP-Glo™ Assay Performance Using Four Different Sources of ATP.

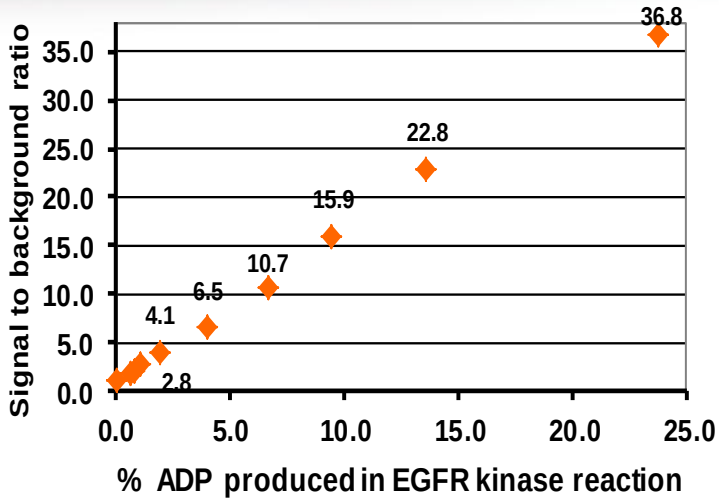
	1mM			100µM			10µM		
% ADP in ATP	50%	25%	12.5%	50%	25%	12.5%	50%	25%	12.5%
Pro	54	32	18	37	20	10			
Comp	35	21	11	31	16	8	13	8	5%
Competitor T	15	10	5	16	9	5	13	7	4
Competitor G	16	10	6	18	10	6	12	6	4

New Ultrapure ATP

ADP-Glo™ sensitivity is highly improved with the new Ultra Pure ATP

ADP-Glo™ detection of Tyrosine kinase activity





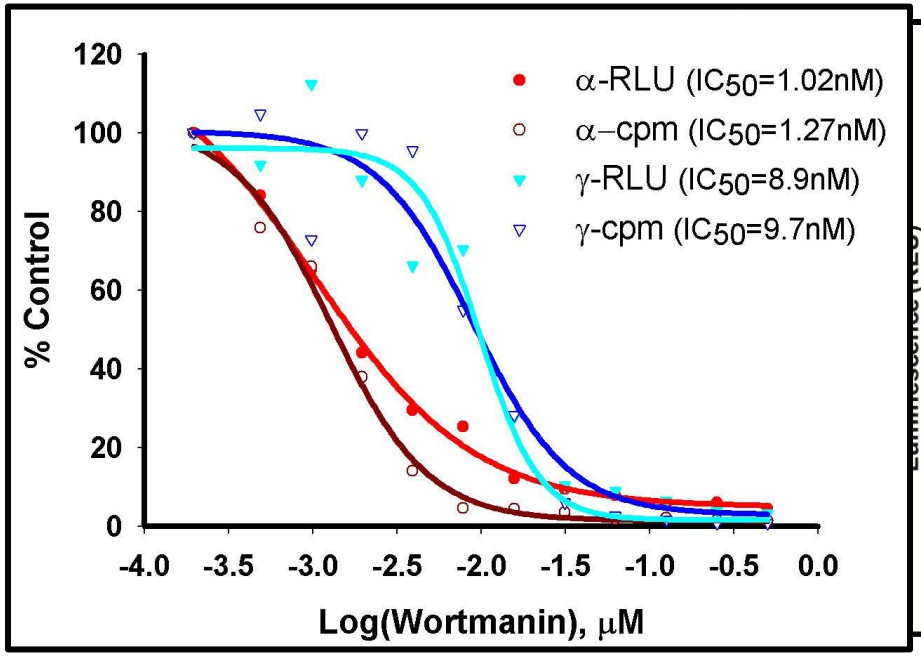
EC50

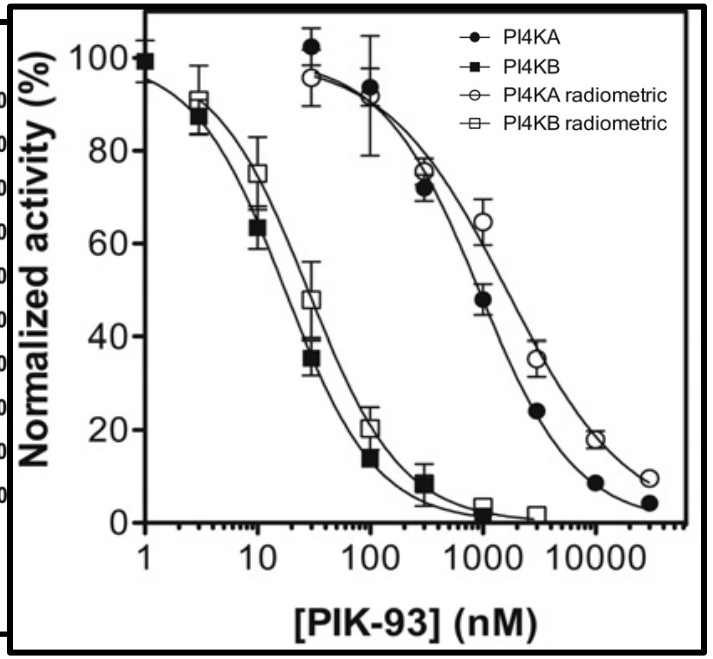
EGFRL858R (ng/reaction)	100	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.1	0.05	0
RLU (Average)	27753	28278	26871	24358	18944	13551	8384	5849	3923	2404	1502	1045	368
% ADP produced	87.4	89.1	84.6	76.7	39.8	23.8	13.6	9.5	6.7	4.0	1.9	1.1	0
S/B	75.4	76.8	73.0	66.2	51.5	36.8	22.8	15.9	10.7	6.5	4.1	2.8	1

	SB_{10}
EGFR (ng)	0.36

Amount of enzyme to generate 5-10% conversion

Comparison between ADP Glo and Radioactivity Assay





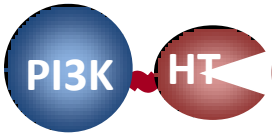
Vidugiriene J, et al (2009). Evaluating the utility of a bioluminescent ADP-detecting assay for lipid kinases. Assay Drug Dev Technol. 7(6):585-97.

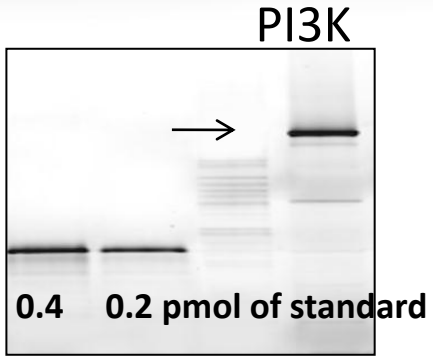
Tai AW, Bojjireddy N, Balla T. (2011) A homogeneous and nonisotopic assay for phosphatidylinositol 4-kinases. Anal Biochem. 417(1):97-102.

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

Validation of ADP-Glo with immobilized Kinase

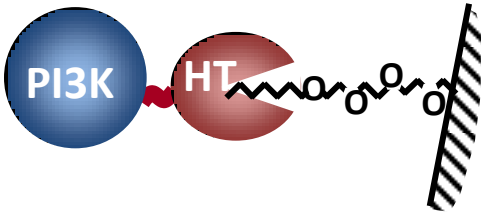
Expression as HaloTag fusion (HT)



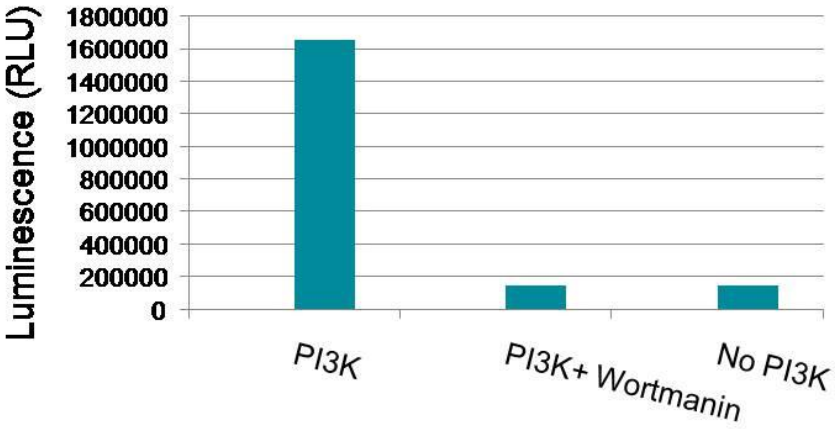


In gel Visualization

Immobilization on HaloLink beads

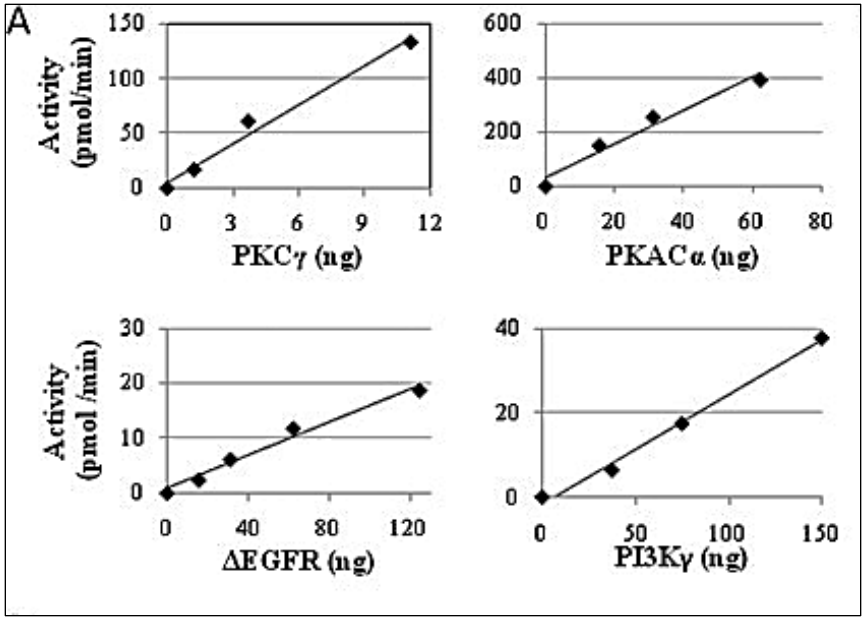


Activity measurement **ADP-Glo**



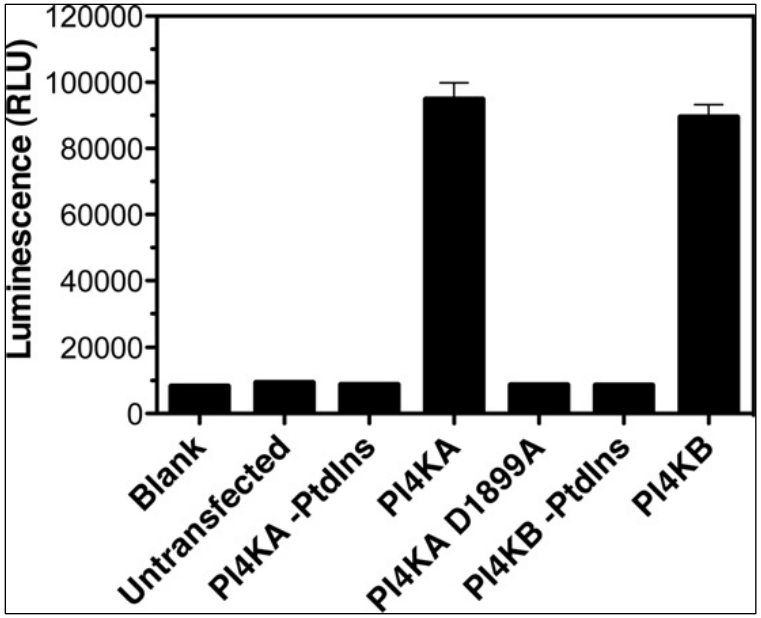
Validation of ADP-Glo with mammalian expressed Kinases

HaloTag



Ohana RF, et al (2011) HaloTag-based purification of functional human kinases from mammalian cells. Protein Expr Purif.76(2):154-64.

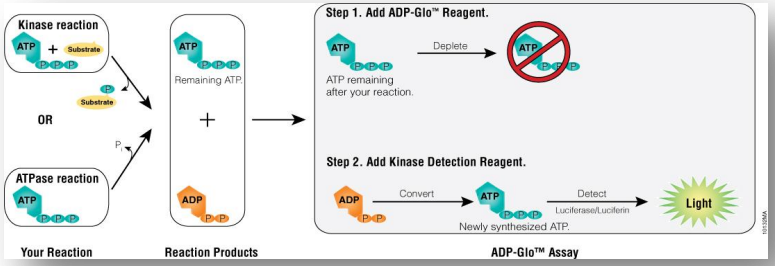
FLAG Tag



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

One assay platform - many applications

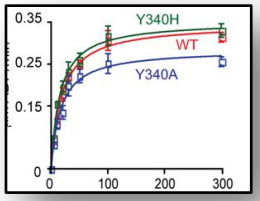
ADP-Glo™ is a Universal *in vitro* Biochemical Assay for all types of Kinase Studies



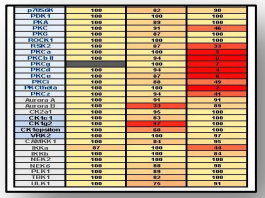
ADP-Glo™ Assay Platform

High-Throughput Screening

Mode of action studies

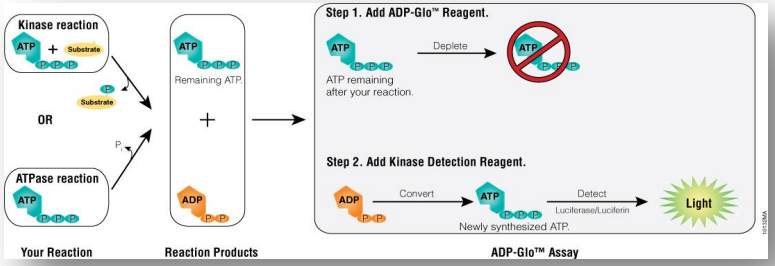


Kinase inhibitor profiling



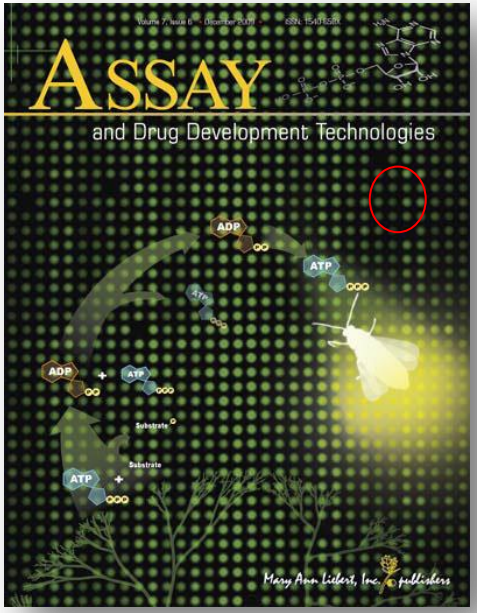
One assay platform - many applications

Screening Kinase Inhibitors with ADP-Glo™ Kinase assay



ADP-Glo™ Assay Platform

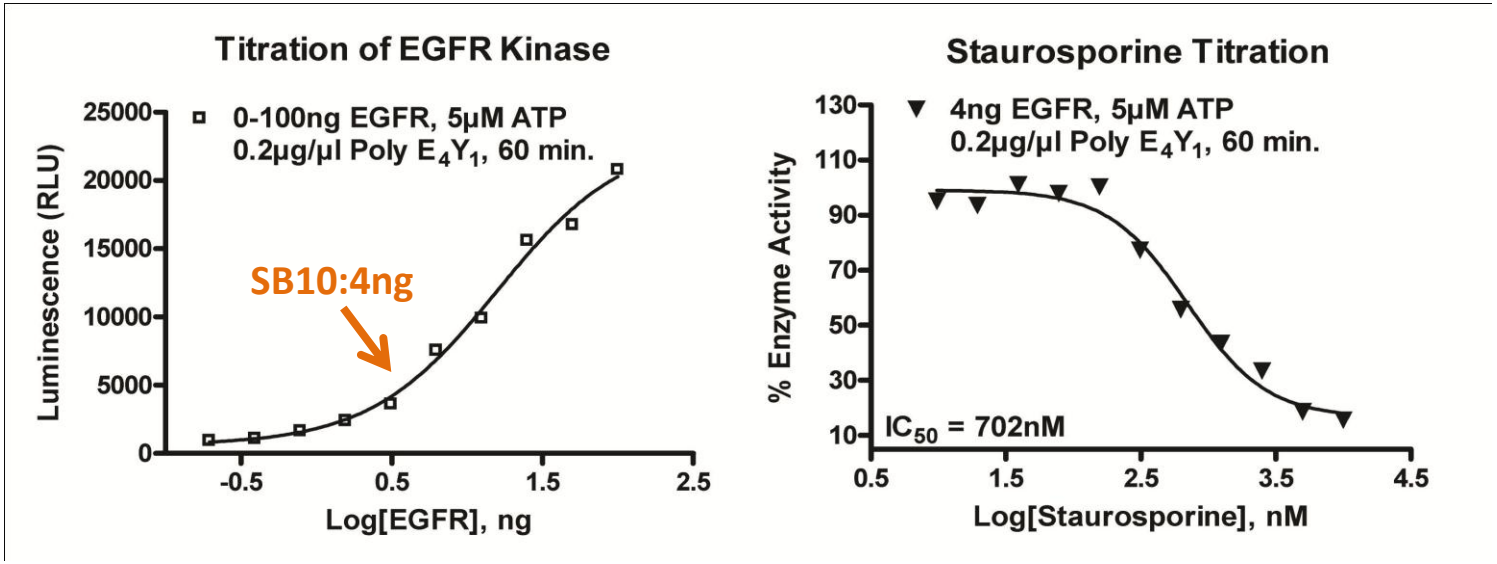
High-Throughput Screening



Free Access here:
http://www.liebertonline.com/toc/adt/7/6

Developing kinase assay for screening and profiling

SB 10 value allows a high dynamic range with less variability and more accuracy in an inhibitor screening or Profiling (High Z' factor)

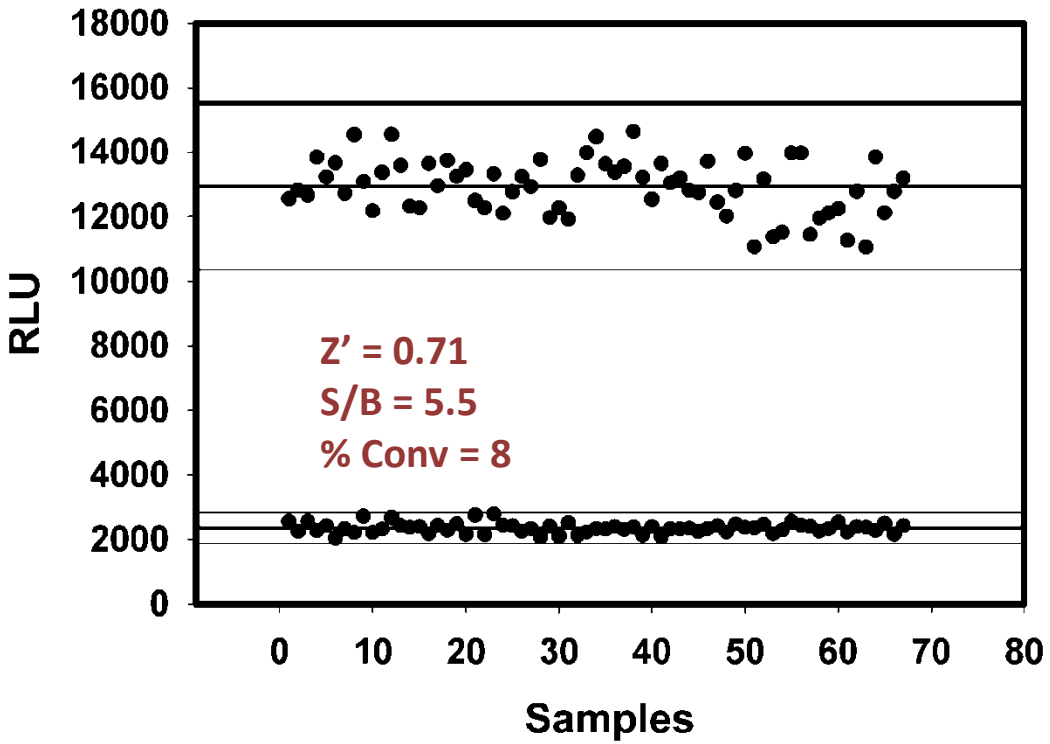


Kinase Titration: Using the defined ATP concentrations, perform ½ serial dilution of Enzyme.

SB10 value: Corresponds to the amount of Kinase needed to generate a 5-10% substrate conversion.

Sensitivity, Robustness of the ADP-Glo™ Assay

During HTS, a Z' values >0.5 shows a robust assay



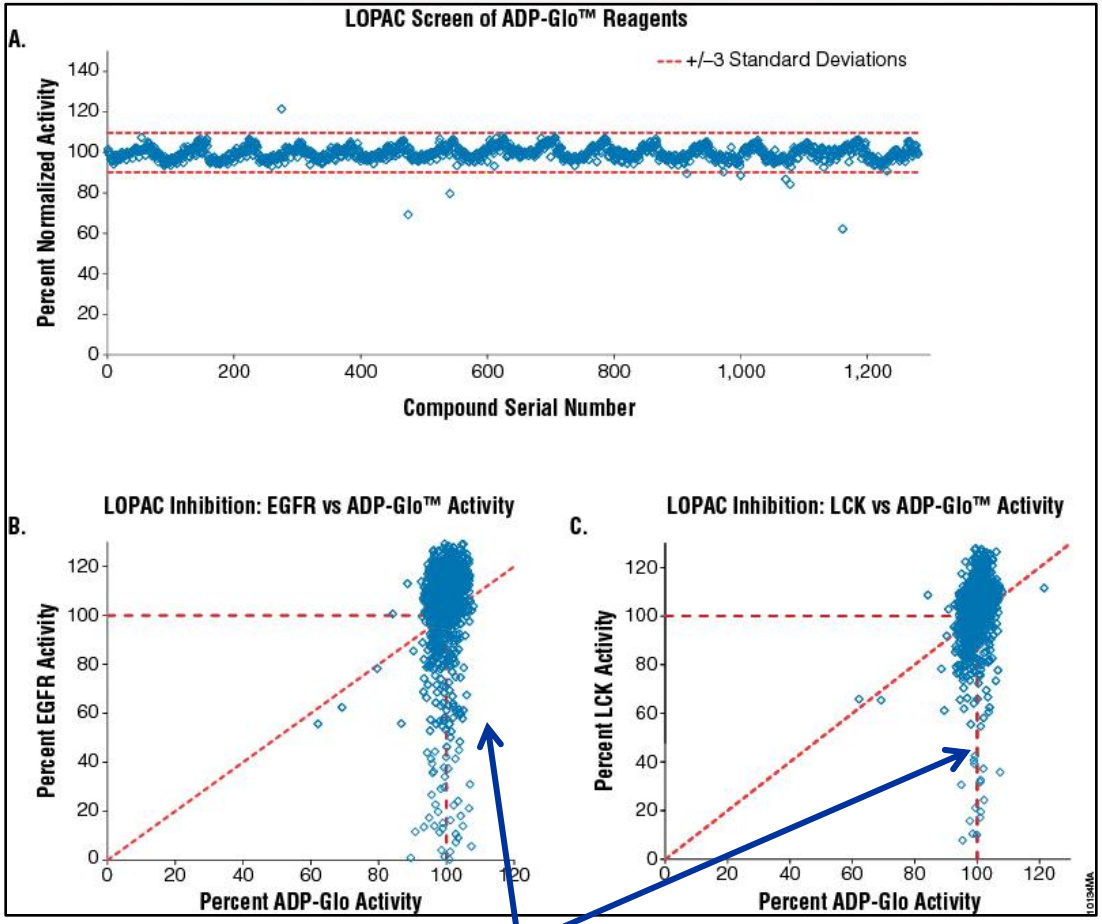
$$\text{Z-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}.$$

σ_p: STDEV of sample σ_n: STDEV of control
μ_p: Mean of sample μ_p: Mean of control

Zhang JH, Chung TD, Oldenburg KR (1999) *J Biomol Screen.* **4**, 67–73

ADP-Glo™ can detect low Enzyme activity with a high Z' value

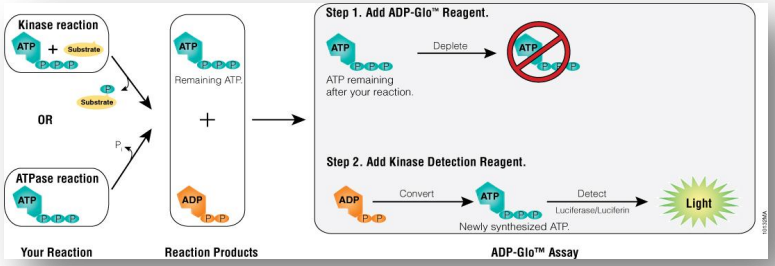
Screening for Kinase inhibitors using luminescent ADP detection



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

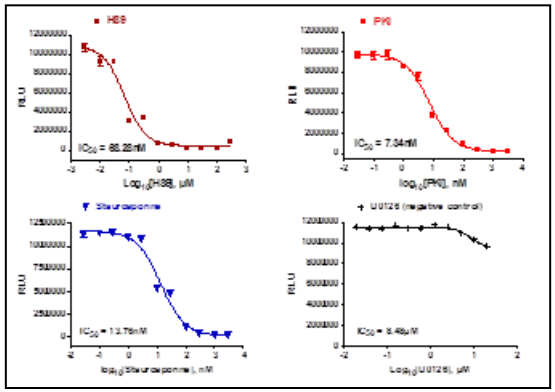
One assay platform - many applications

Mode of Action Studies with ADP-Glo™ Kinase Assay



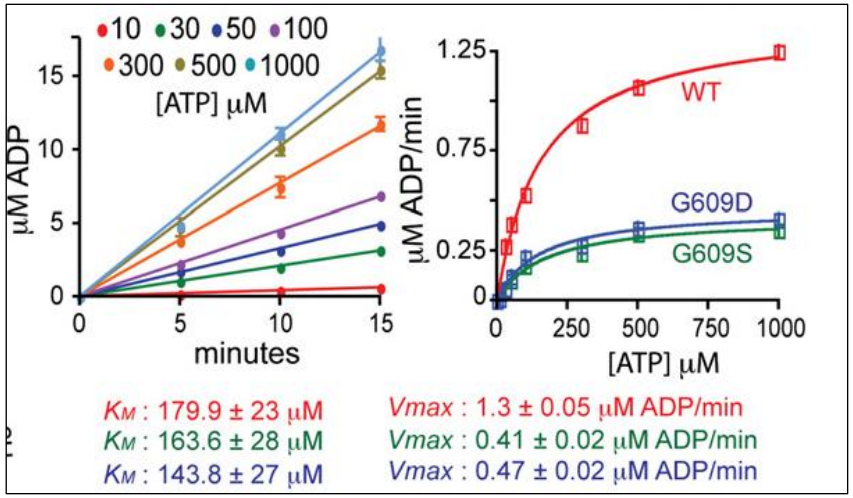
ADP-Glo™ Assay Platform

Mode of action studies

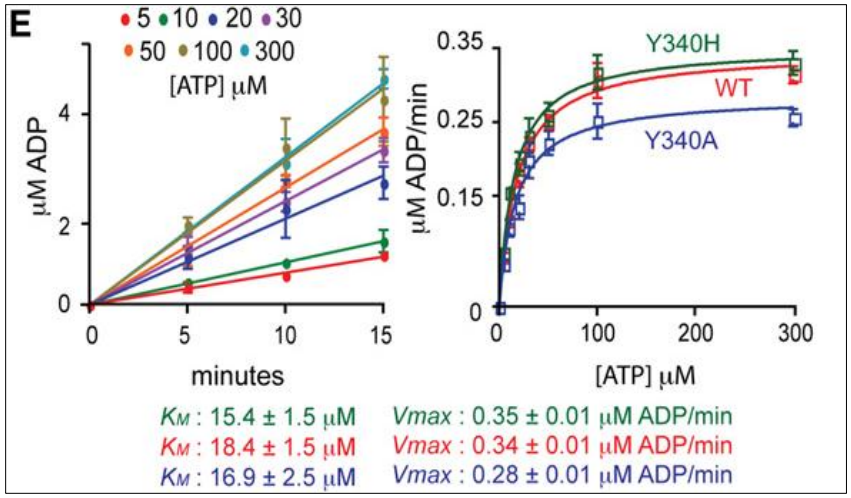


Determination of Biochemical values using ADP-Glo™

C-Src



Haspin

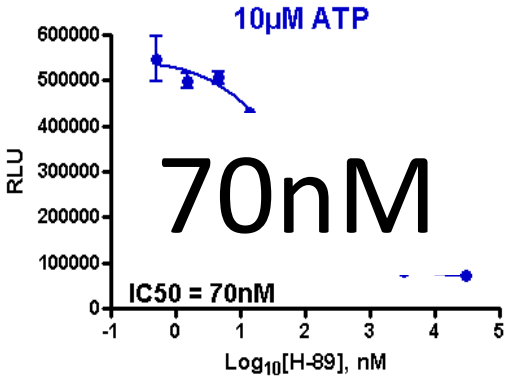


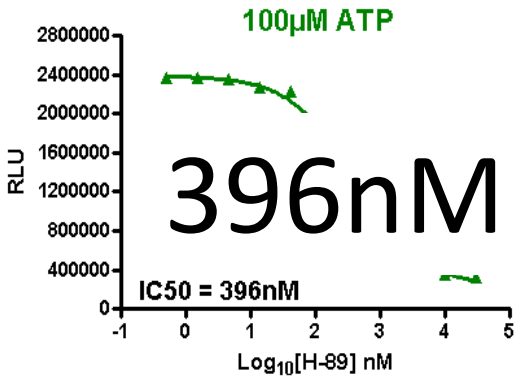
Balzano D et al., (2011). A general framework for inhibitor resistance in protein kinases. Chem Biol. 18(8):966-75.

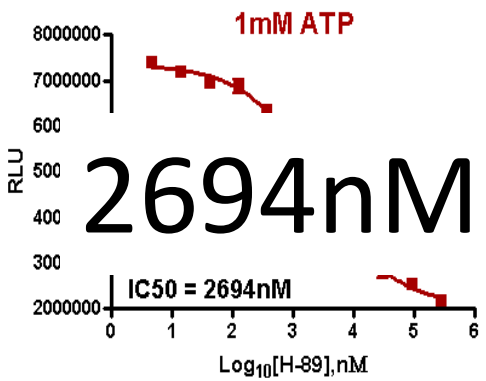
ADP-Glo ™produces Km values similar to literature

Determination of inhibitor's mechanism of action

PKA ATP Competitive inhibitor H-89

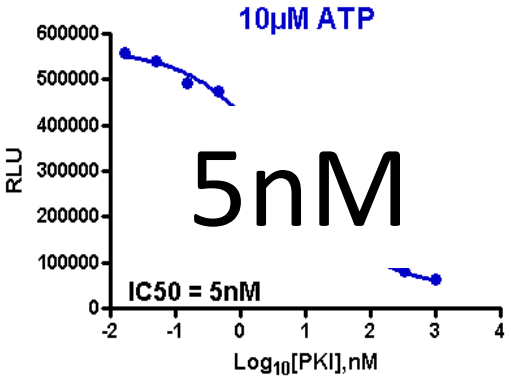


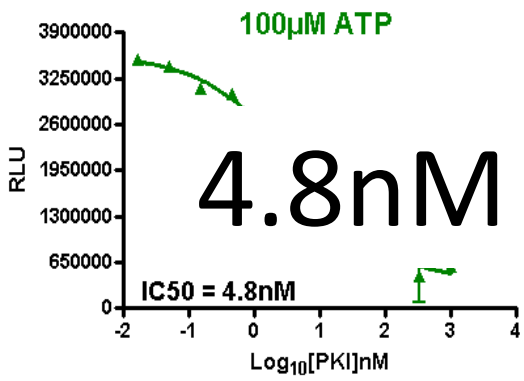


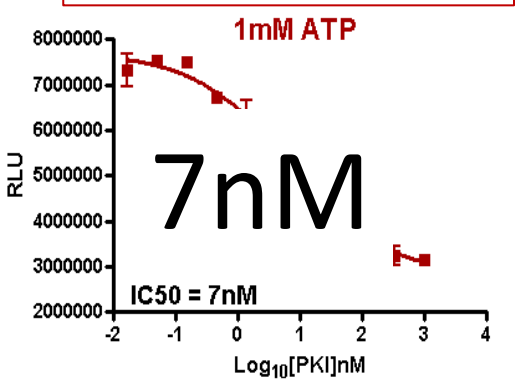


PKA ATP non Competitive inhibitor PKI

Cellular Levels mM



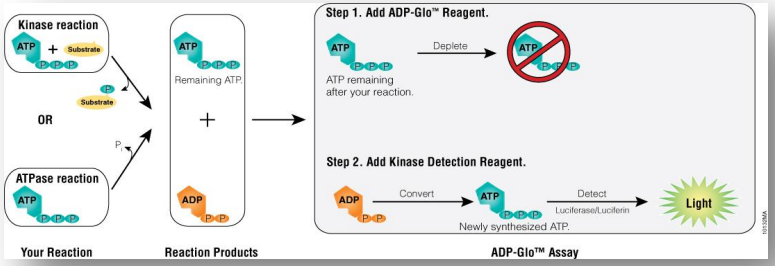




ADP-Glo™ is a perfect assay to distinguish between ATP competitive and non competitive kinase inhibitors

One assay platform - many applications

Profiling Kinase Inhibitors with ADP-Glo™ Kinase Assay



ADP-Glo™ Assay Platform

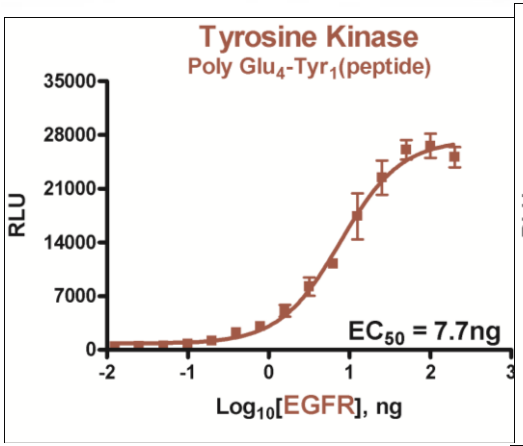
Kinase inhibitor profiling

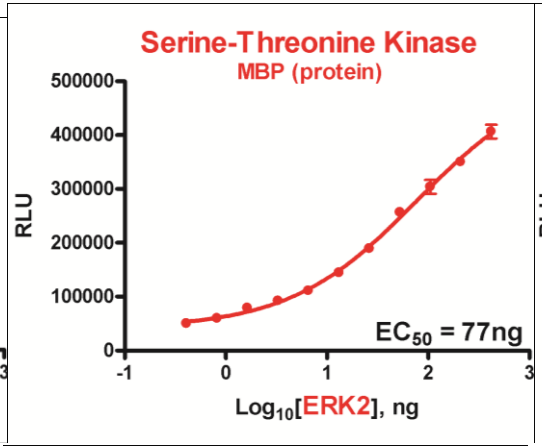
Kinase	LCK inhibitor	CDK/CRK inhibitor	Ro-32-0432
ASK1	100	83	99
HPK1	100	21	92
KHS1	96	77	100
MEK1	100	89	99
MEKK1	64	82	100
MINK1	100	96	81
NIK	100	92	100
MEKK2	60	95	95
MST1	92	63	98
MYO3b	100	91	95
PAK1/CDC42	66	100	100
PAK3	100	90	100
SLK	91	85	93
TAOK1	92	25	100
TNIK	88	100	94
ERK2	81	100	85
GSK3b	68	20	25
JNK1	92	100	100
JNK3	91	98	100
p38a	100	100	100
p38g	100	100	100
p38d	98	100	100
CDK2/A2	12	1	73
CDK3/E1	45	17	80
CDK5/p25	76	2	98
CDK5/p35	73	1	100
CDK6/D3	58	100	60
CDK9/K	37	13	63
CLK3	57	100	94
AMPK A1/B1/G1	100	68	71
AMPK A1/B1/G2	100	67	100
AMPK A2/B1/G1	100	46	100
CAMK2a	100	81	100
CAMK2g	100	72	100
CAMK4	100	67	100
DAPK1	100	100	100
STK33	99	100	100
CHK2	100	100	100
MAPKAPK2	100	100	100
MARK1	100	73	100
PASK	100	87	100
PKCmu	100	93	100

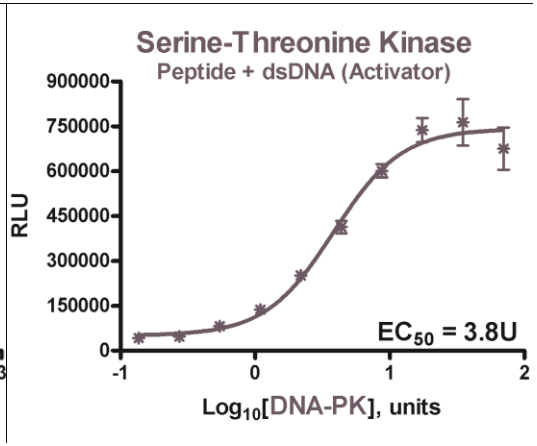
Diverse kinase-substrate combinations with ADP-Glo™

Promega

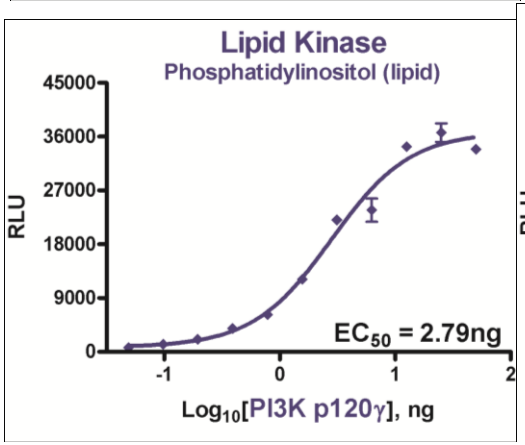
Protein Kinases

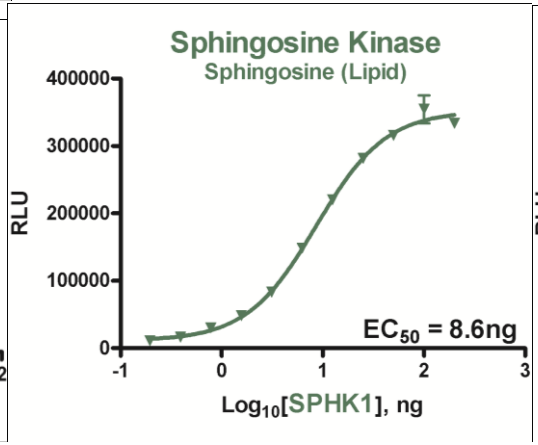


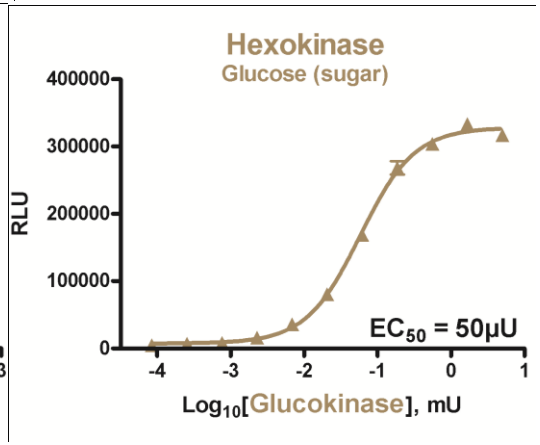




Non-Classical Kinases

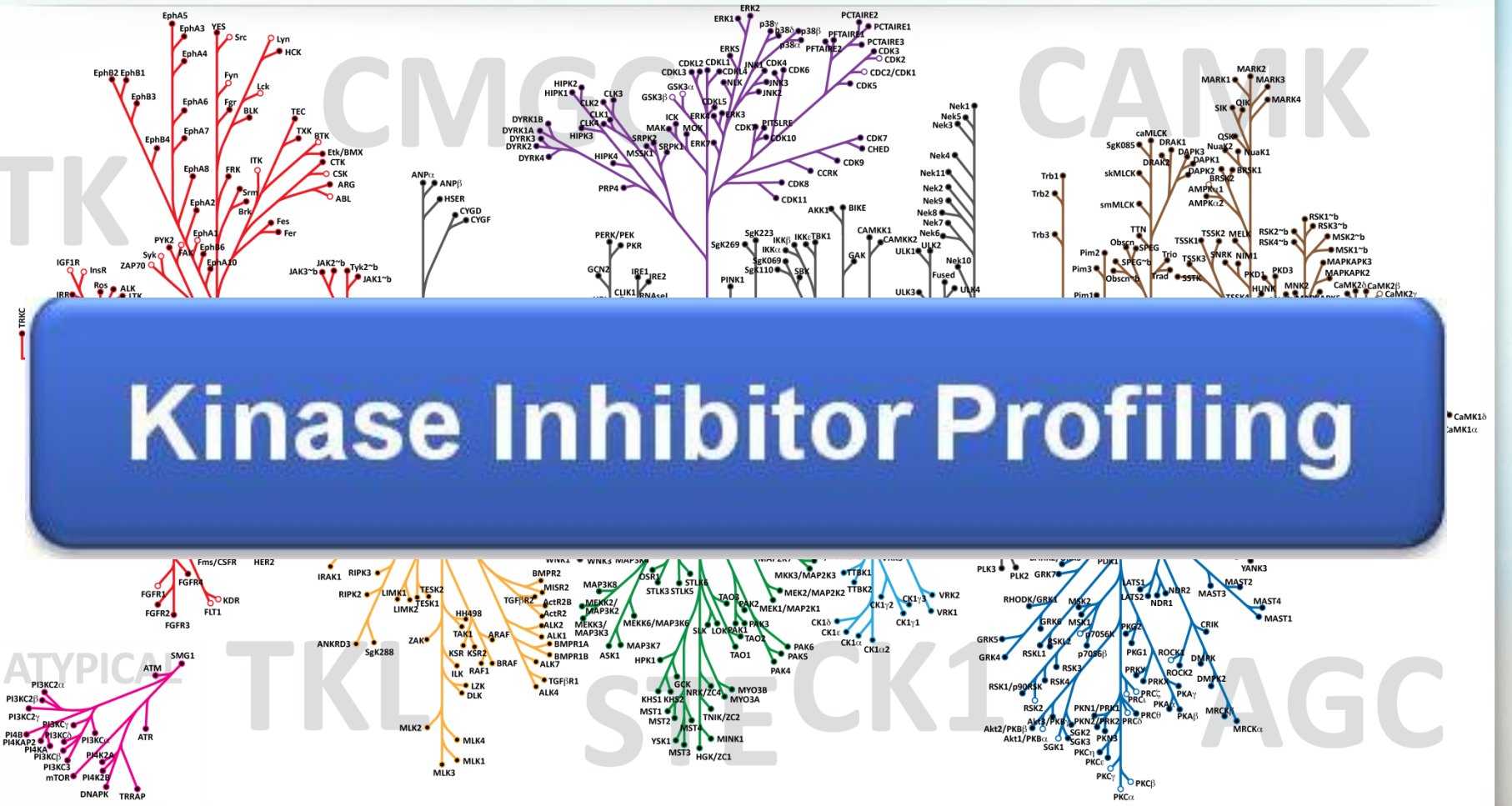






ADP-Glo™ Kinase Assay detects the activity of any Kinase regardless of the substrate chemical structure

Profiling the Human Kinome



ADP-Glo™ is a universal Kinase Assay that detects the activity of any Kinase regardless of the substrate chemical structure

Profiling Kinase inhibitors is a critical step in Drug Development

Assessing selectivity and cross reactivity of Kinase inhibitors

Knowing the exact kinase inhibition profile of a compound is critical for the understanding of its mode of action and to assess any side effects that may result from its extra activities.

Gleevec (BCR-ABL), PD 98059 (p38), U0126 (MEK1), Rapamycin (mTOR), were shown to be very selective inhibitors, while PP1 and PP2 (Src family) inhibit other kinases in different families, CSK, SAPK2a/p38, CK1, KIT and BCR-ABL.

Identifying new targets during a compound profiling could lead to novel therapeutic applications.

Gleevec (BCR-ABL inhibitor) was marketed by Novartis initially for the treatment of chronic myeloid leukemia (CML), then its indication was expanded to gastrointestinal stromal tumors (GIST) because of its effect on c-Kit kinase.

Profiling already in development or clinically approved compounds against drug resistant Kinase targets could lead to new treatment of relapsed patients with ineffective first-line targeted therapies.

MK-0457 (Aurora Kinase inhibitor) in clinical development for the treatment of solid tumors is shown to inhibit an ABL kinase mutant (T315I), that it could be potentially used to treat Gleevec-resistant cancer.

Providing Complete solutions for the kinase field:

Promega + Signalchem collaboration

Kinase Enzyme Systems (KES)

Kinase Enzyme System (KES) offering

2 catalog numbers per enzyme system

Promega
Catalog # V9101

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
Promega Catalog #

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

=

Promega
Catalog # V9061

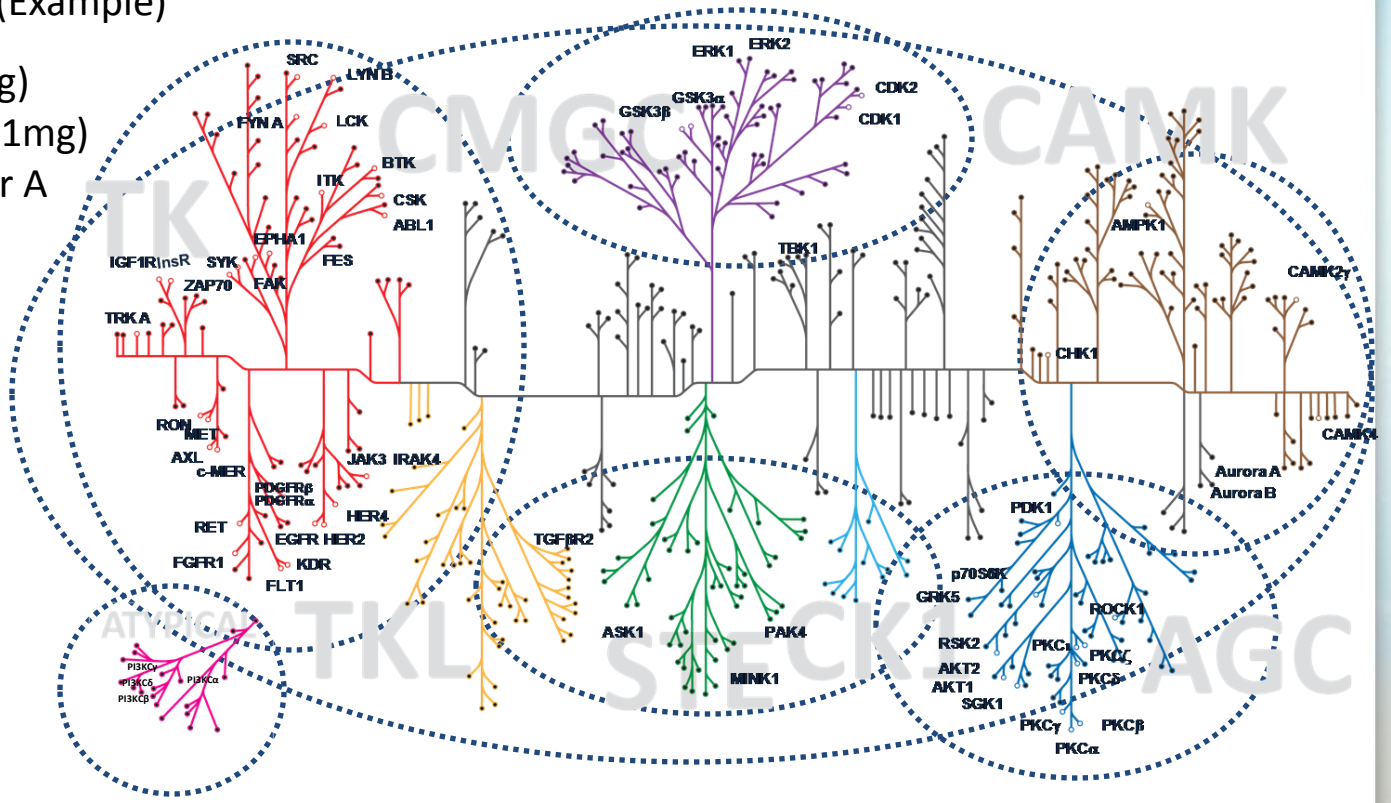
Promega kinase panel
(Kinase Enzyme Systems)

Kinase Enzyme Systems (Example)

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

+

ADP-Glo™ Kinase Assay



Promega Kinase Panel contains initially 70 Kinases that Cover the Human kinome

Validating Kinase Enzyme systems with ADP-Glo™

Promega

Kinases were tested with ADP-Glo™ Kinase Assay

Kinase	Substrate	ATP Concentration (µM)	SB10 (ng)	IC50 Staurosporine (nM)
CMGC				
CDK1/CyclinA2	Histone H1	50	1	1.9
CDK2/CyclinA2	Histone H1	50	0.6	1.8
CDK5/p25	Histone H1	10	2	1
CDK5/p35	Histone H1	10	3	1.2
ERK1	MBP	50	2.8	>1000
ERK2	MBP	50	1.6	>1000
GSK3a	GSK3 Substrate	25	1	6.4
GSK3b	GSK3 Substrate	25	1	0.3
LYN B	SRC substrate	25	3	4.7
MET	Poly (Glu_4, Tyr_1)	10	4	280
PDGFRa	Poly (Glu_4, Tyr_1)	25	7	0.48
PDGFRb	Poly (Glu_4, Tyr_1)	25	6.5	0.3
RET	IGF1tide	25	2	2.3
RON	Axltide	25	2	76.5
SRC	SRC substrate	50	1.8	250.8
SYK	Poly (Glu_4, Tyr_1)	10	2.8	0.17
TRKA	Poly (Glu_4, Tyr_1)	50	0.6	0.3
ZAP70	Poly (Glu_4, Tyr_1)	3	2.9	103.5
TKL				
IRAK4	MBP	25	5.0	0.75
TGFβR2	MBP	50	30	>1000
STE				
ASK1	MBP	25	4.4	21.4
MINK1	MBP	50	0.6	2.8
PAK4	Akt substrate II	5	10	0.2
p70S6K	S6K substrate	25	20	82
PDK1	PDKtide	5	8	0.75
PKCa	CREBtide	25	0.2	5.94
PKCb II	CREBtide	50	0.1	6.18
PKCd	CREBtide	50	0.8	1.87
PKCg	PKCtide	50	0.3	6.2
PKCi	CREBtide	25	0.5	518
PKCz	CREBtide	5	0.2	>1000
ROCK1	S6K substrate	5	10	6.7
RSK2	RSK Substrate	25	1	11.3
SGK1	Akt (PKB) substrate	50	3.5	12.2
OTHER				
Aurora A	MBP	25	7	0.47
Aurora B	MBP	25	6.4	1.5
NEK2	MBP	50	5	>1000
PLK1	Dephospho Casein	5	40 (SB4)	>1000
TBK1	MBP	25	2.2	0.05
ULK1	MBP	10	5.0	18.6

- ADP-Gl … ted
- To gene …

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

Enzymes are offered separately
&
together in a complete Class I profiling kit

+

PI:PS Lipid Kinase substrate 0.5ml x 1mM

or

PIP2:PS Lipid Kinase substrate 0.25ml x 1mM

Promega
Catalog #XXXX

ADP-Glo™ Kinase Assay

Promega kinase panel
(Kinase Enzyme Systems)

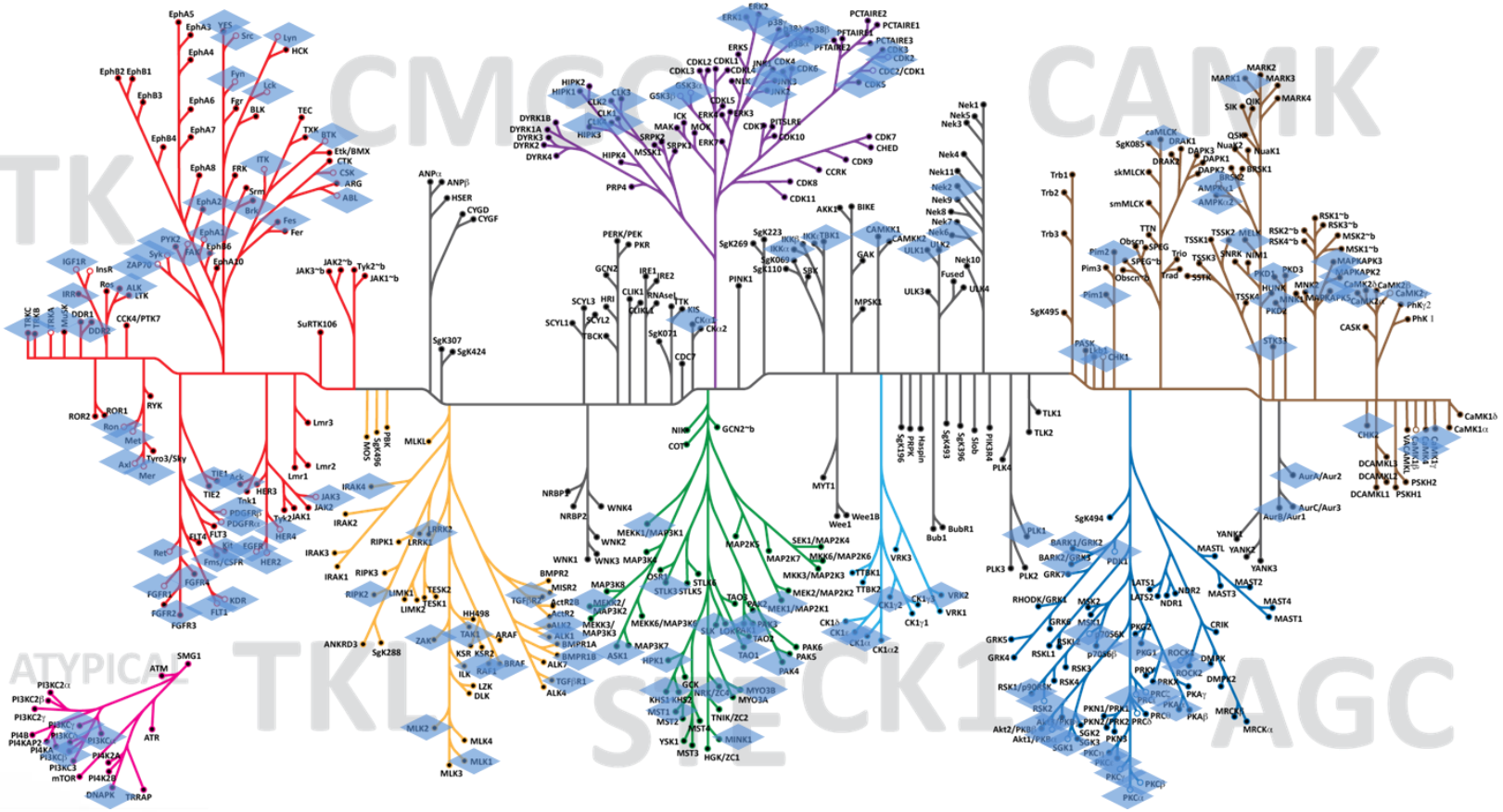
Promega Kinase Panel contains a biologically diverse set of kinases that cover the human kinome

TK			TKL	CAMK	STE	CMGC	AGC	OTHER/CK1
ABL1	RET	KIT T670I	IRAK4	AMPK A1/B1/G1	ASK1	CDK1/CyclinA2	AKT1	Aurora A
AXL	RON	PDGFRA D842V	TGFβR2	CAMK2g	MINK1	CDK2/CyclinA2	AKT2	Aurora B
BTK	SRC	PDGFRA T674I	TGFbR1	CAMK4	PAK4	CDK5/p25	GRK5	NEK2
c-MER	SYK	RET Y791F	BRAF	CHK1	MEK1	CDK5/p35	p70S6K	PLK1
CSK	TRKA	RET V804L	BRAF V600E	AMPK A2/B1/G1	PAK3	ERK1	PDK1	TBK1
EGFR	ZAP70	MET M1250T	RAF1(EE)	MAPKAPK5	MST1	ERK2	PKCa	ULK1
EPHA1	FLT3	FGFR3 K650E	MLK2	PIM2	SLK	GSK3a	PKCb II	NEK6
FAK	FMS	FLT3 D835Y	TOPK	PKD2	HPK1	GSK3b	PKCd	DNA-PK
FES	FGFR2		ALK1	PIM1	PAK1/CDC42	JNK3	PKCg	IKKa
FGFR1	FGFR4		MLK1	CAMK1g	MEKK1	p38a	PKCi	CK2a1
FLT1	ACK		RIPK2	MLCK/MYLK	MEKK2	p38g	PKCz	CAMKK1
FYN A	DDR2		BMPR1b (ALK6)	CAMK2a	MYO3b	CDK9/Cyclin K	ROCK1	CK1α1
HER2	PYK2		TAK1-TAB1	MAPKAPK2	STK39/STLK3	JNK1	SGK1	VRK2
HER4	c-Kit		ALK2	MAPKAPK3	KHS1	CDK2/Cyclin E1	RSK2	CK1g2
IGF1R	TRKB		ZAK	DAPK1	NIK	p38b	AKT3	CK1epsilon
InsR	BRK		LRRK2	SIK	TAOK1	p38d	PKCe	CK2
ITK	EGFR L858R			MYLK3/caMLCK		CDK7	RSK1	
JAK3	EGFR L861Q			STK33		CDK4/Cyclin D3	PKCtheta	
KDR	EGFR T790M			CHK2		CDK6/Cyclin D3	ROCK2	
LCK	EGFR T790M L858R			PKCmu (PKD1)		CDK3/CyclinE1	p70S6Kb	
LYN B	ABL1 E255K			PASK		CLK1	GRK2	
MET	ABL1 G250E			MELK		CLK3	PKG	
PDGFRa	ABL1 T315I			MARK1		HIPK1	PKA	
PDGFRb	ABL1 Y253F			AMPK A1/B1/G2		HIPK3	PKC	

www.promega.com/kinase

Promega kinase panel

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



Broad Human Kinome coverage with 174 Kinase Enzyme Systems

Promega kinase profiling strips for flexible and targeted inhibitor profiling

Important kinase targets organized by kinase families

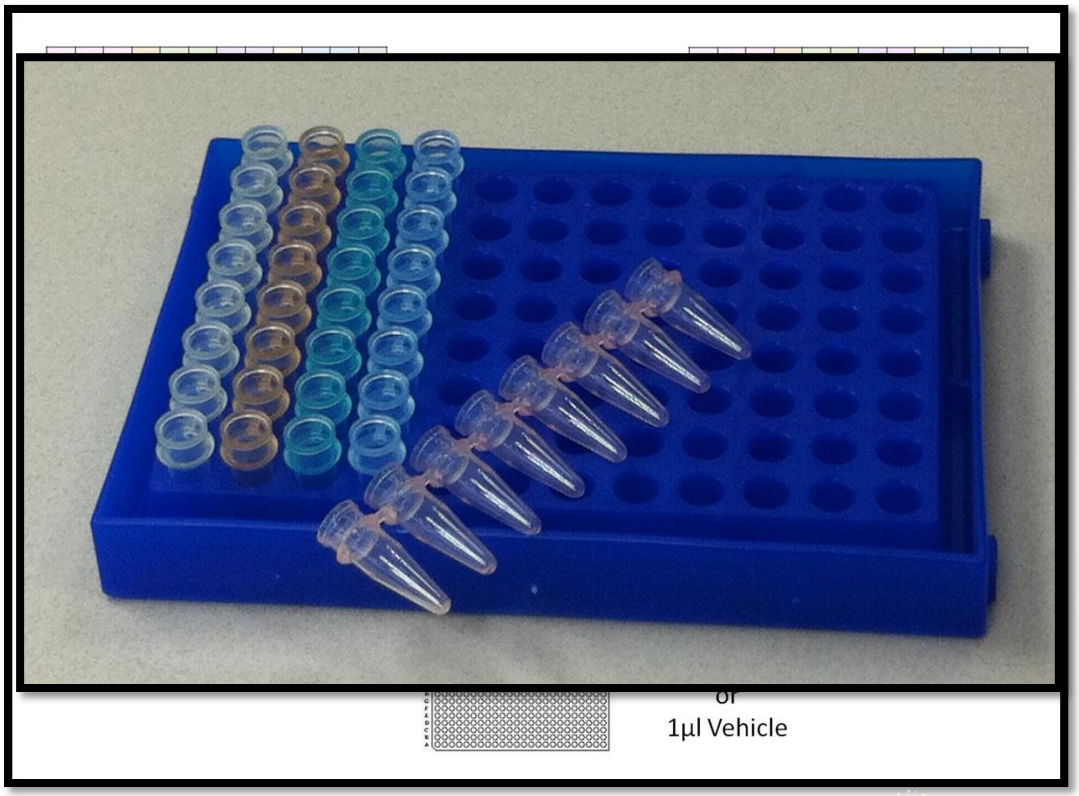
1	2	3	4	5	6	7	8
EGFR	ABL1	AXL	FGFR1	BMPR1b	ALK1	ASK1	MEKK2
HER2	BRK	EPHA1	FGFR2	BRAF	ALK2	HPK1	MST1
HER4	BTK	FAK	FGFR4	IRAK4	BRAF V600E	KHS1	MYO3b
IGF1R	CSK	ITK	FLT1	LRRK2	MLK2	MEK1	PAK1
InsR	FYN A	JAK3	FLT3	MLK1	RIPK2	MEKK1	PAK3
KDR	LCK	PYK2	FMS	RAF1(EE)	TGFβR2	MINK1	SLK
PDGFRα	LYN B	SYK	MET	TAK1-TAB1	TOPK	NIK	TAOK1
PDGFRβ	SRC	TRKA	RET	TGFbR1	ZAK	PAK4	TNIK

9	10	11	12	13	14	15	16
ERK2	CDK1/ CyclinA2	AMPK A1/B1/G1	CHK1	AKT1	PKCα	Aurora A	CAMKK1
GSK3β	CDK2/ CyclinA2	AMPK A1/B1/G2	CHK2	p70S6K	PKCβ II	Aurora B	IKKα
JNK1	CDK3/ CyclinE1	AMPK A2/B1/G1	MAPKAP K2	PDK1	PKCγ	CK2α1	IKKβ
JNK3	CDK5/ p25	CAMK2a	MARK1	PKA	PKCδ	DNA-PK	NEK2
p38α	CDK5/ p35	CAMK2g	MELK	PKC	PKCε	CK1α1	NEK6
p38β	CDK6/ Cyclin D3	CAMK4	PASK	PKG	PKCι	CK1γ2	PLK1
p38γ	CDK9/ Cyclin K	DAPK1	PIM1	ROCK1	PKCθ	CK1ε	TBK1
p38δ	CLK3	STK33	PKCμ (PKD1)	RSK2	PKCζ	VRK2	ULK1

16 Kinase strips (128 Kinases)

Streamlined profiling protocol

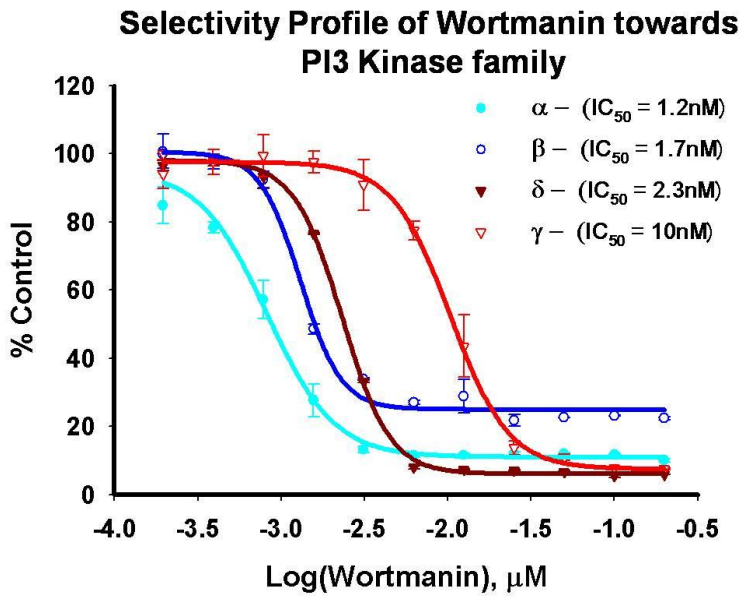
Profiling Kinase strips



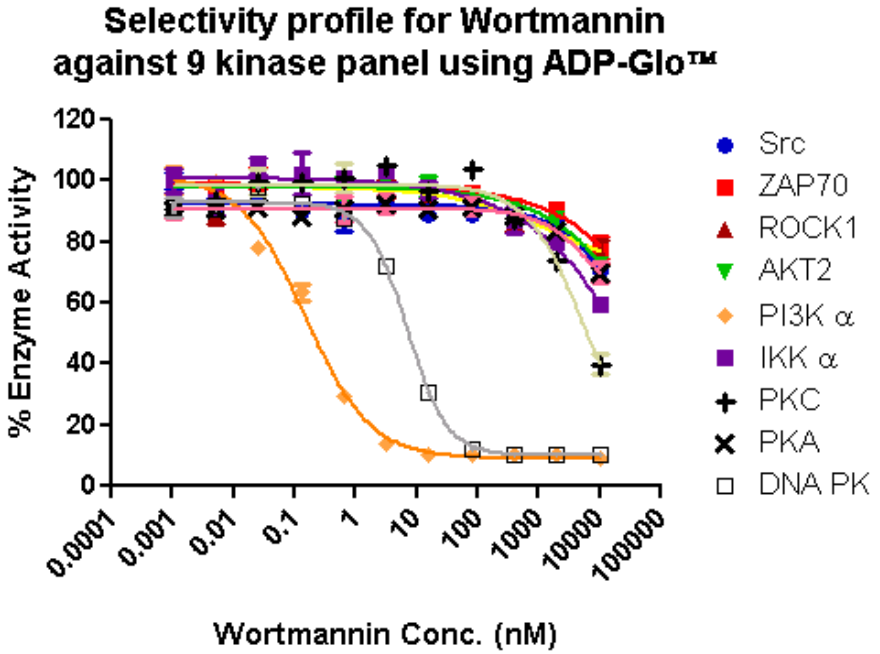
Profiling with ADP-Glo platform made simple

Profiling kinase inhibitors with ADP-Glo™ kinase platform

Against a kinase family



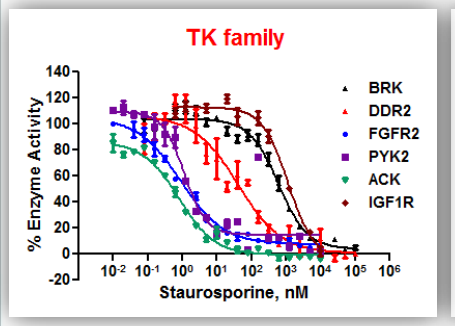
Against a Panel of Different families

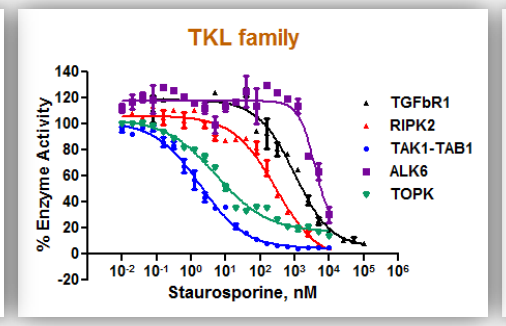


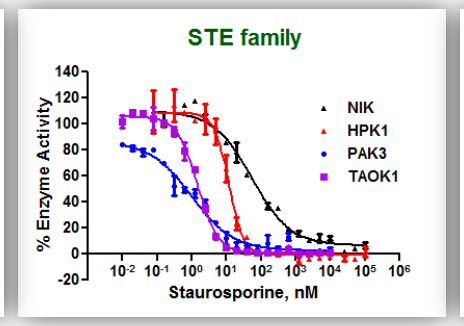
As a "One Assay for all", ADP-Glo™ is an ideal assay for Profiling inhibitors

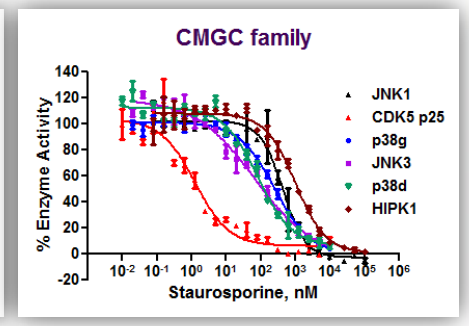
Profiling kinase inhibitors with ADP-Glo™ kinase platform

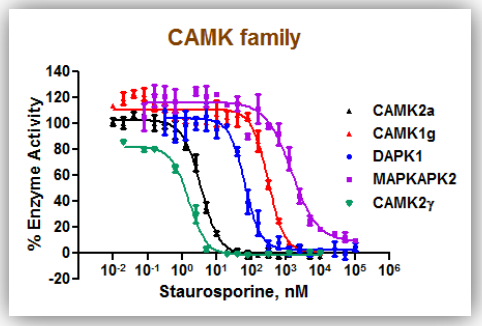
Staurosporine selectivity profiles of small kinase family panels

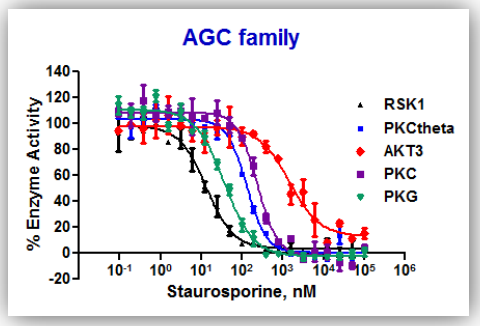


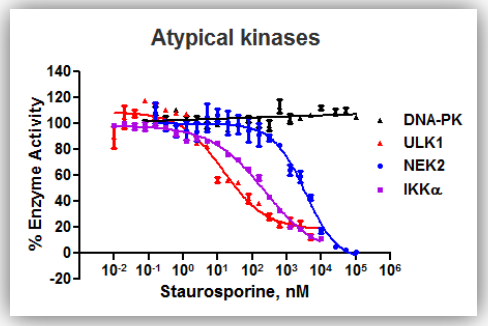












"One Assay for all", ADP-Glo™ is ideal for profiling inhibitors against large or small kinase panels

Determination of inhibitor profiles of different kinase families

LCK inhibitor (Src family specific)

Kinase	LCK inhibitor	CDK/CRK inhibitor	Ro-32-0432
HER2	72	78	100
HER4	16	83	100
IGF1R	100	100	100
Insr	95	96	100
KDR	56	98	96
PDGFRa	25	77	97
PDGFRb	36	63	63
ABL1	[illegible]	100	59
BRK	1	100	72
BTK	3	87	88
CSK	11	100	80
FYN A	4	66	90
LCK	20	89	81
LYN B	12	96	71
SRC	15	92	100
AXL	[illegible]	94	100
EPHA1	68	85	98
FAK	100	100	100
ITK	84	86	90
JAK3	100	92	92
PYK2	100	90	100
SYK	89	96	95
TRKA	36	100	50
FGFR1	38	27	98
FGFR2	76	96	98
FGFR4	81	93	100
FLT1	84	91	100
FLT3	13	93	75
FMS	62	100	100
MET	87	71	100
RET	17	99	100
BMPR1b	100	87	100
BRAF	98	98	100
IRAK4	100	92	100
LRRK2	71	84	100
MLK1	96	72	100
RAF1(EE)	92	92	100
TAK1-TAB1	100	72	100
TGFbR1	100	76	100
ALK1	95	99	86
BRAF V600E	97	100	97
MLK2	87	90	100
RIPK2	15	95	87
TGFβR2	82	100	100
TOPK	99	100	100
ZAK	87	100	100

Cdk/Crk inhibitor (CDK family specific)

Kinase	LCK inhibitor	CDK/CRK inhibitor	Ro-32-0432
ASK1	100	83	99
HPK1	100	21	92
KHS1	96	77	100
MEK1	100	89	99
MEKK1	64	82	100
MINK1	100	96	81
NIK	100	92	100
MEKK2	60	95	95
MST1	92	63	98
MYO3b	100	91	95
PAK1/CDC42	66	100	100
PAK3	100	90	100
SLK	91	85	93
TAOK1	92	25	100
TNIK	88	100	94
ERK2	81	100	85
GSK3b	68	20	25
JNK1	92	100	100
JNK3	91	98	100
p38a	100	100	100
p38g	100	100	100
p38d	98	[illegible]	100
CDK2/A2	12	1	73
CDK3/E1	46	17	80
CDK5/p25	76	2	98
CDK5/p35	73	1	100
CDK6/D3	58	100	60
CDK9/K	37	13	63
CLK3	57	[illegible]	94
AMPK A1/B1/G1	100	68	71
AMPK A1/B1/G2	100	67	100
AMPK A2/B1/G1	100	46	100
CAMK2a	100	81	100
CAMK2g	100	72	100
CAMK4	100	67	100
DAPK1	100	100	100
STK33	99	100	100
CHK2	100	100	100
MAPKAPK2	100	100	100
MARK1	100	73	100
PASK	100	87	100
PKCmu	100	93	100

Ro-32-0432 (PKC family specific)

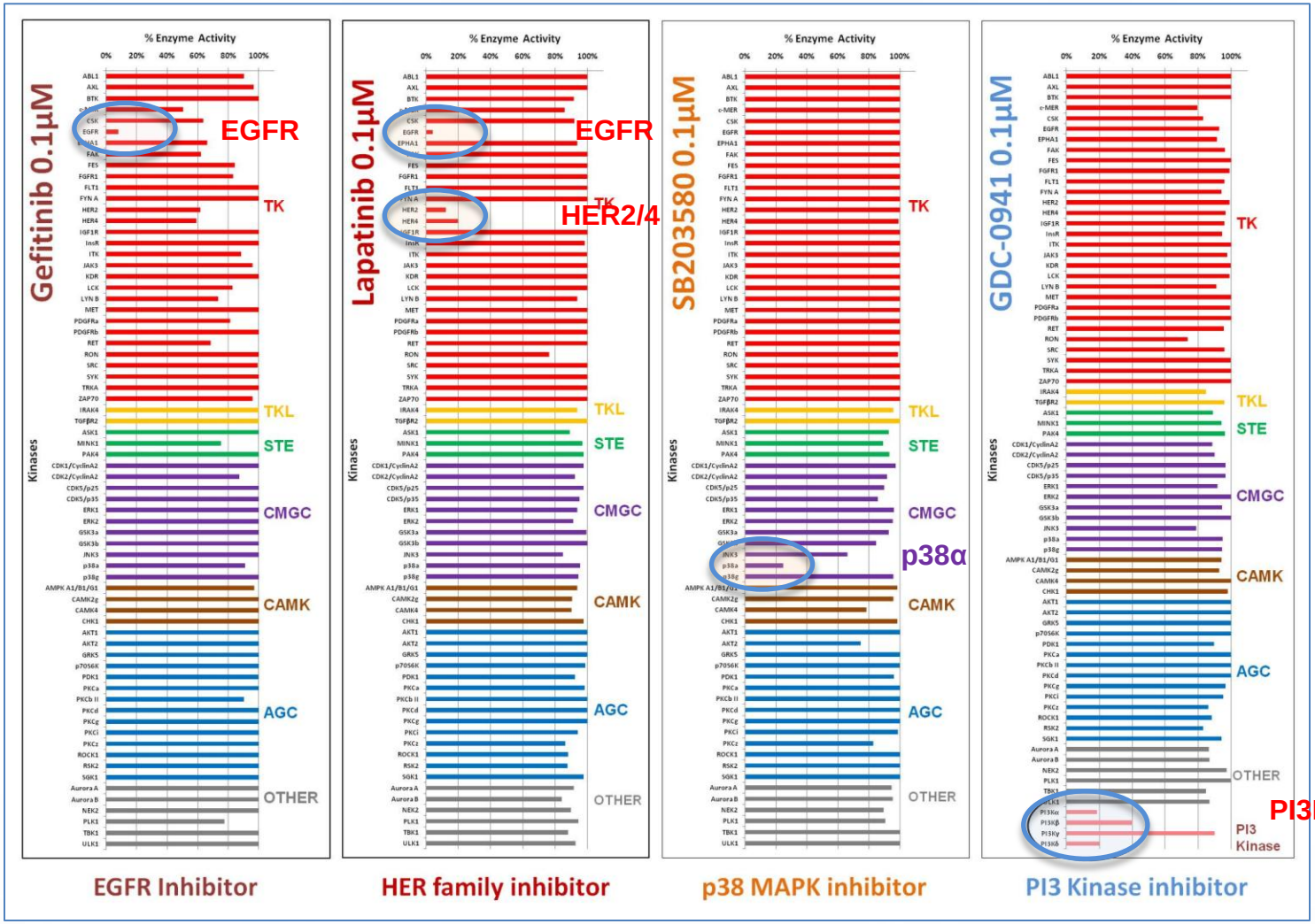
Kinase	LCK inhibitor	CDK/CRK inhibitor	Ro-32-0432
p70S6K	100	82	90
PDK1	100	100	100
PKA	100	89	10[illegible]
PKC	100	91	[illegible]
PKG	100	87	1[illegible]
ROCK1	100	100	100
RSK2	100	87	33
PKCa	100	100	5
PKCb II	100	94	0
PKCg	[illegible]	100	7
PKCd	100	94	4
PKCe	100	87	6
PKCi	100	88	49
PKCtheta	100	100	2
PKCz	100	94	41
Aurora A	100	91	91
Aurora B	100	33	89
CK2a1	100	95	100
CK1a1	100	83	100
CK1g2	100	17	100
CK1epsilon	100	60	100
VRK2	100	100	97
CAMKK1	100	84	95
IKKa	87	100	44
IKKb	100	100	84
NEK2	100	100	100
NEK6	100	88	98
PLK1	100	89	100
TBK1	100	82	100
ULK1	100	75	91

More inhibited

Less inhibited

ADP-Glo™ Kinase platform for convenient and meaningful selectivity profiles creation

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



ADP-Glo™ Assay in peer review

Assay and Drug development Technologies Journal (December 09 issue) contains
6 publications related to ADP-Glo:

1. **ADP-Glo Technology description**
2. **ADP-Glo for Lipid Kinases**
3. **uHTS screening with ADP-Glo**
4. **Comparison of ADP-Glo with other assays**
5. **Radioactivity vs. ADP-Glo**
6. **Profiling with ADP-Glo**

Free Access here:
http://www.liebertonline.com/toc/adt/7/6

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 .

General features of ADP-Glo™ assay

- ✓ Homogenous, non radioactive and Antibody Free
- ✓ Luminescent assay: Less Compound interference
- ✓ Stable luminescent signal: Batch plate processing
- ✓ Universal: Any kinase/any substrate, ideal for profiling
- ✓ Robust Assay (Z' higher than 0.7)
- ✓ High dynamic range: High Signal to Background at low % ATP to ADP conversion allows use of lower amount of enzyme during HTS
- ✓ Broad range of ATP conc. (linear from low to high range) allows distinction between ATP competitive and non competitive inhibitors
- ✓ High sensitivity: 20nM ADP detected with more than 2.5 fold difference

ADP-Glo™ assay 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

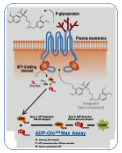
ADP-Glo™ Platform

1. ADP-Glo™ Kinase Assay

Kinase reactions up to 1mM ATP

2. Kinase Enzyme Systems

174 complete kinase assays



3. ADP-Glo™ Max Assay

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

Next

4. Complete solutions for Profiling

Large number of kinases ready to assay in a flexible "do it yourself" profiling kits

Thank you

Questions?

hicham.zegzouti@promega.com

Promega