Comprehensive Protein Kinase Profiling Panel for Selective Inhibitor Screening provided by Carna Biosciences "The Kinase company"

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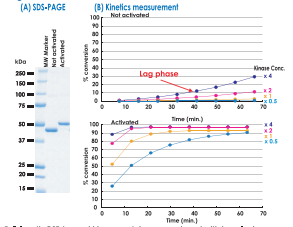
Abstract

Kinase selectivity profiling has become an invaluable tool to assess the potential of kinase inhibitors in the drug discovery process. To support drug discovery programs at biotechnology and pharmaceutical companies, a Japanese biotechnology company, Carna provides 255 active protein kinases and profiling services against over 200 kinases under its optimized assay conditions. In this presentation, we describe a new kinase profiling panel using an off-chip mobility shift assays leveraged on the Caliper LifeSciences' LabChip® 3000 (LC3000) systems. Peptide substrates were identified for approximately 200 kinases. The peak-separation conditions of the fluorescence-labeled substrates and their phosphorylated products were optimized. Carna' s active kinases phosphorylated their peptide substrates on dose- and time-dependent manners. The Km value for ATP was determined for each kinase. Consequently more than 150 kinase assays are available in the profiling panel on the LC3000. These kinases belong to TK, TKL, STE, CK1, AGC, CMGC, and CAMK families in the kinome. Therefore, our kinase profiling panel is useful for optimizing the lead compounds.

Procedure *Off-Chip mobility shift assay for profiling*

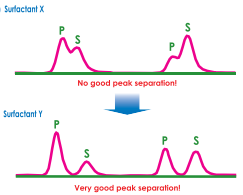
Characterization of recombinant kinase (Fig. 1).
Optimization of peak separation (Fig. 2).
Km determination (Fig. 3 and Fig. 4).
Rerationship between % conversion and IC_{50} (Fig. 5).
IC_{50} determination (Fig. 6 and Specificity profiles for clinical kinase inhibitors).

Fig. 1 Characterization of recombinant kinase



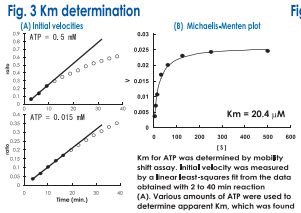
Full-length GST-tagged kinase protein was produced with baculovirus -infected insect cells. Kinases were purified with several steps chromatography (without any contaminated kinase derived from host cell). Characterization of recombinant protein kinase by SDS-PAGE (A) and kinetic measurement of non activated kinase (B, top) and activated kinase after activation by auto-phosphorylation or upstream kinases (B, bottom).

Fig. 2 Optimization of peak separation



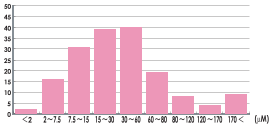
Peak separation is most importance for analysis on the mobility shift assay. Figures show peak separation condition used (A) surfactant X (B) surfactant Y in running buffer. Peaks show ratio of substrate to phosphorylated product from same wells. We examined that peak separation was deemed acceptable for use, conditions for validation were optimized.

Fig. 3 Km determination



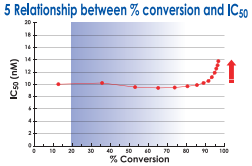
Km for ATP was determined by mobility shift assay. Initial velocity was measured by a linear least-squares fit from the data obtained with 2 to 40 min reaction (A). Various amounts of ATP were used to determine apparent Km, which was found to be 20.4 μM (B).

Fig. 4 Distribution of Km values for ATP



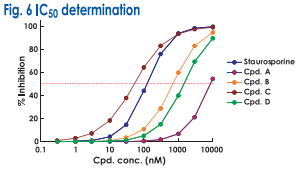
Km values for ATP were determined by mobility shift assay. Km values were collected from 167 kinases.

Fig. 5 Relationship between % conversion and IC_{50}



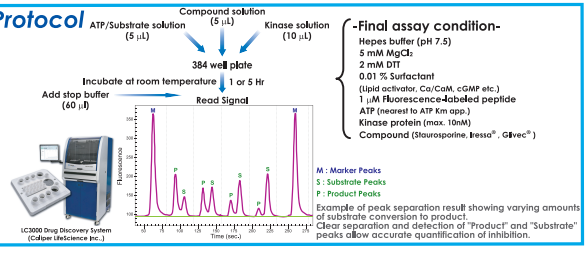
To evaluate kinase inhibition, it is important to use a kinase concentration which is lower than IC_{50}/100 values of reference compound (max. 10nM) Blue zone indicate optimal % conversion ratio area for IC_{50} values evaluation.

Fig. 6 IC_{50} determination



Representative data of IC_{50} values of several chemical compounds. Dose response curves for 5 compounds assayed against a Tyr kinase. The ATP was used at the concentrations around the Km. IC_{50} values of staurosporine, Cpd.A, B, C, D were 120, 8700, 692, 52.2 and 1440 nM, respectively.

Protocol

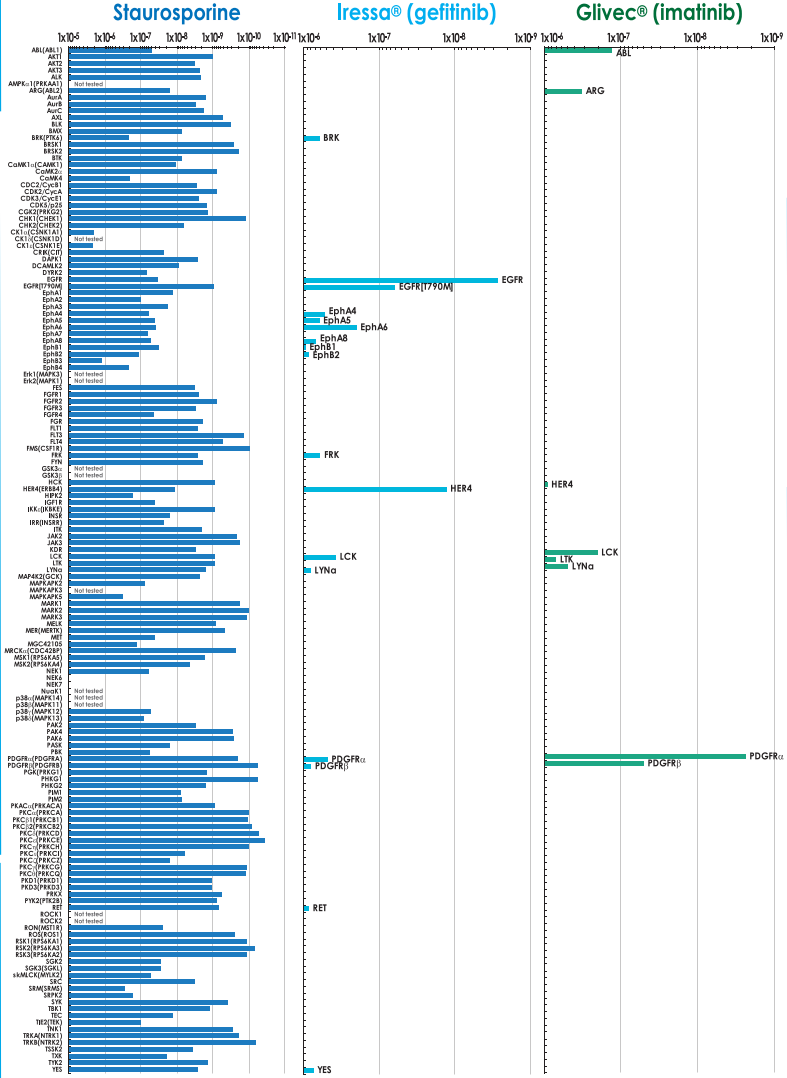


-Final assay condition-

Hepes buffer (pH 7.5)
5 mM $MgCl_2$
2 mM DTT
0.01 % Surfactant
(Lipid activator, Ca/CaM, cGMP etc.)
1 μM Fluorescence-labeled peptide
ATP (nearest to ATP Km app.)
Kinase protein (max. 10nM)
Compound (Staurosporine, Iressa®, Glivec®)

Example of peak separation result showing varying amounts of substrate conversion to product. Clear separation and detection of "Product" and "Substrate" peaks allow accurate quantification of inhibition.

Specificity profiles for clinical kinase inhibitors (IC_{50}, M)



151 kinases (62 tyr kinases, 1 drug-resistant EGFR[T790M] and 88 ser/thr kinases) for specificity profiling were picked up from kinase list. Profilings were conducted at 10 concentrations (10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001 and 0.0003 μM) in duplicate for each compound.

Conclusion

Carna Biosciences has developed assays for kinase profiling panel using mobility shift assay, LabChip® microfluidic platform, which is applicable to various kinases.

We have obtained the high reproducibility of kinase selectivity profiling by Iressa® and Glivec® which can prove the accuracy of new assay platform.

Profiling panel with 167 kinases (71 TKs and 96 STKs) has been developed by using Mobility Shift Assay!

Kinases represented in this presentation are shown in kinome tree, as well as in Kinase List.

Kinase List

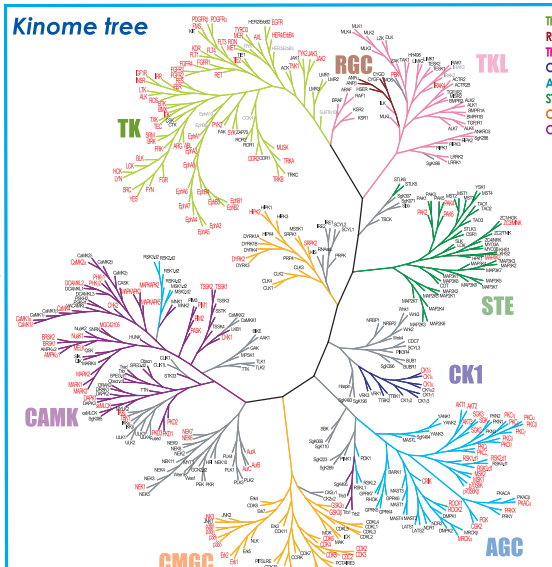
Tyr kinase

Cytoplasmic			Receptor			
ABL	BLK	BTK	ALK	EPHA3	FGFR3	ROS
ABL[T315I]	BRK	ITK	LTK	EPHA4	FGFR4	TIE2
ARG	FGR	TEC	AXL	EPHA5	IGF1R	TRKA
TNK1	FRK	TXK	MER	EPHA6	INSR	TRKB
PYK2	FYN		TYRO3	EPHA7	IRR	FLT1
FES	HCK		DDR2	EPHA8	MET	FLT4
FER	LCK		EGFR	EPHB1	RON	KDR
JAK2	LYNa		EGFR[T790M]	EPHB2	FLT3	
JAK3	LYNb		MUSK	EPHB3	FMS	
TYK2	SRC		HER4	EPHB4	PDGFRα	
SRM	SYK		EPHA1	FGFR1	PDGFRβ	
YES	BMX		EPHA2	FGFR2	RET	

Ser/Thr kinase

AGC group			CAMK group			CMGC group			Other group	CK1 group
AKT1	PKCβ2	CRIK	CaMK1α	MGC42105	PIM2	CDC2/Cyc81	Erk1		AurA	CK1α
AKT2	PKCγ	RSK1	CaMK1δ	Nuak1	PKD1	CDK2/CycA	Erk2		AurB	CK1δ
AKT3	PKCδ	RSK2	CaMK2α	PASK	PKD2	CDK3/CycE1	p38α		AurC	CK1ε
MRCKα	PKCε	RSK3	CaMK4	DAPK1	PKD3	CDK4/CycD3	p38β		IKKε	
MSK1	PKCζ	SGK	AMPKα1	DCAMKL2	CHK2	CDK5/p25	p38γ		TBK1	STE group
MSK2	PKCθ	SGK2	BRSK1	MAPKAPK2	TSSK1	CDK6/CycD3	p38δ		NEK1	MAP4K2
CGK2	PKCη	SGK3	BRSK2	MAPKAPK3	TSSK2	DYRK2			NEK6	MINK
PGK	PKCι		CHK1	MAPKAPK5		HIPK2			NEK7	PAK2
PKACα	p70S6K		MARK1	skMLCK		SRPK2			PBK	PAK4
PRKX	p70S6Kβ		MARK2	PHKG1		GSK3α			IRAK4	PAK6
PKCα	ROCK1		MARK3	PHKG2		GSK3β				
PKCβ1	ROCK2		MELK	PIM1		JNK3				

Kinome tree



TK, tyrosine kinases
RGC, receptor guanylate cyclase
TKL, tyrosine kinase-like kinases
CK1, casein kinase family
AGC, containing PKA, PKG, PKC families
STE, homologs of yeast sterile 11, sterile 20 kinases
CMGC, containing CDK, MAPK, GSK3, CLK families
CAMK, calcium/calmodulin dependent kinases.

"Carna" is a goddess in Roman mythology who protects human health. This image is the symbol of Carna Biosciences Inc.,

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