WP029 Kinase Profiling with Homogeneous Assay Platform

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Abstract

Protein kinase signaling pathways are attractive targets for various kinds of human diseases, such as cancer and immune disease. Therefore, protein kinases and their substrates are becoming increasingly important targets for pharmaceutical intervention. In order to obtain good therapeutic results while avoiding adverse effects, it is crucial that the targeted kinase should be specifically and selectively modulated. Profiling technologies clarifies specificity and selectivity of drug candidates. Recently, Carna Biosciences Inc., has completed the collection of the entire (78) catalytically active tyrosine kinases. To date, 75 out of 78 enzymes have been purified, and assessed their activities. Our serine/threonine kinase project was launched with IMAP^{TM} technology, to develop assays to detect the phosphorylation of peptide substrates by fluorescence polarization techniques. This homogeneous assay is a robust analytical platform that can be used for the screening and the optimization of specific/selective kinase activity modulators. Here we present our progress in the development and use of these technologies for tyrosine- and serine/threonine kinase inhibitor profiling.

Introduction

Carna Biosciences Inc., is developing comprehensive tyrosine kinase assay panel for kinase inhibitor specificity profiling with ELISA format. For the next step, we have started developing a profiling system for serine/threonine kinases with homogeneous platforms using IMAP^{TM} technology. Example data of serine/threonine kinase assay in addition to the progress made with tyrosine kinase ELISA are presented.

Method

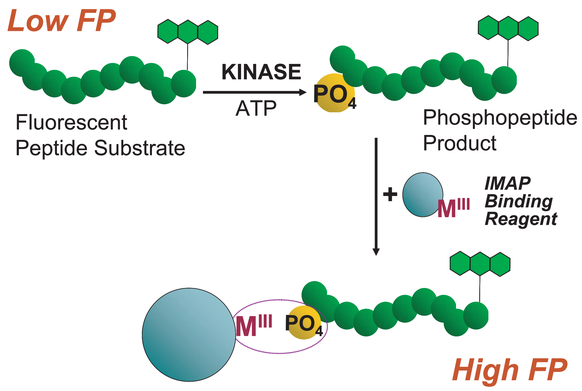

Figure 1. The Principle of IMAP^{TM} technology. The IMAP^{TM} binding reagent (immobilized metal conjugated on nanoparticles) complexes with phospho-peptides on the phosphate group generated in a kinase reaction. Such binding causes a change in the rate of the molecular motion of the peptide, and results in an increase in the FP value observed for the fluorescent label attached at the end of the peptide. This assay is applicable to a wide variety of kinases. This figure and the legend are by courtesy of Molecular Devices Corporation.

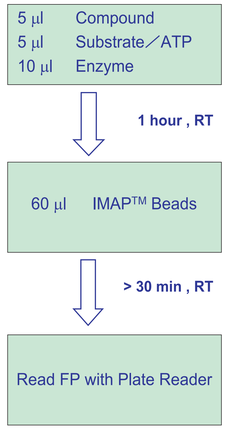

Figure 2. Assay procedure. For kinase reaction, 5 µl compound solution, 5 µl substrate and $MgCl_2$ mixture +/- ATP and 10 µl enzyme solution were incubated 1 hr at RT. By adding 60 µl IMAP^{TM} binding reagent terminated the reaction. After 30 min, FP was measured.

K_m for ATP

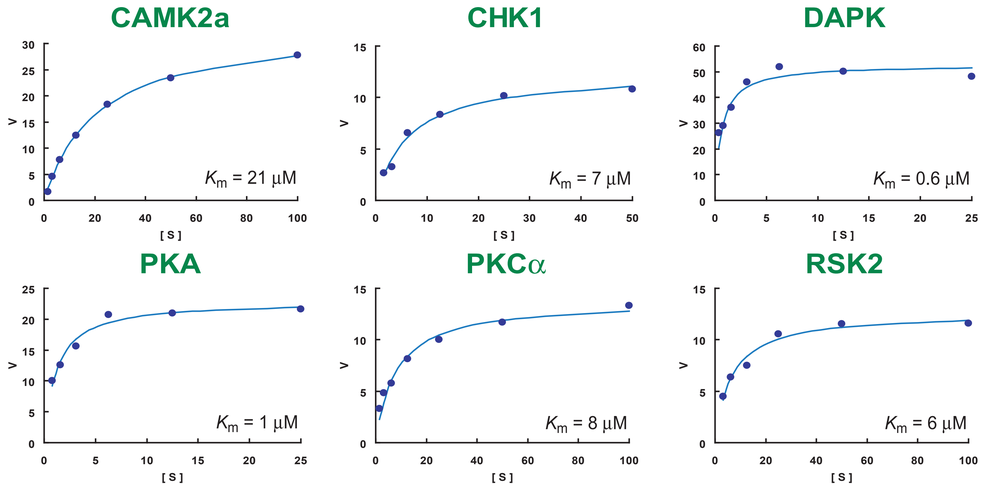

Figure 3. Representative data of Michaelis-Menten plot for ATP. Initial velocity was determined by a linear least-squares fit from the data obtained with 2 to 30 min reaction. ATP concentrations were varied from 0.8 to 100 µM.

IC_{50} Determination (Staurosporine)

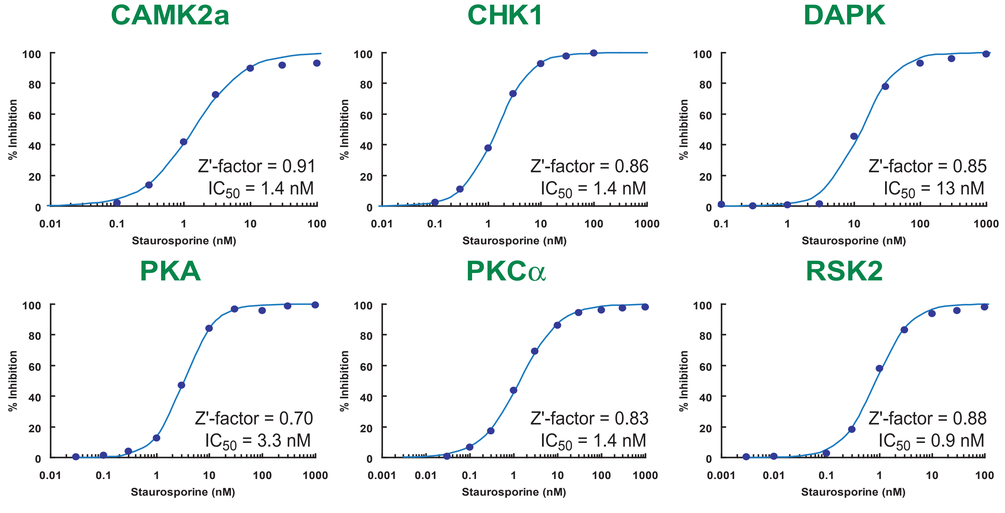

Figure 4. Representative data of IC_{50} values for a kinase inhibitor Staurosporine. The ATP were used at the concentrations around K_m for each kinase. The concentrations of each kinases were lower than 250 ng/mL.

STK IMAP^{TM} Assays in CBS

Table 1. Status of serine/threonine Kinase IMAP^{TM} assay in Carna Biosciences (CBS)

Available		Under Development	
AurA	PKCα	AKT2	JNK2
CRIK	PKCε	CDK2	MAPKAPK2
CaMK2a	PKCγ	Erk1	p38α
CHK1	PKCθ	Erk2	p38β
CHK2	ROCK1	IKKb	PKD2
DAPK1	ROCK2	JNK1	SRPK1
PKA	RSK2		

Currently 14 serine/threonine kinase IMAP^{TM} assay is availble from CBS. Assays for another 12 kinases are under development.

TK ELISA in CBS

Table 2. Status of tyrosine kinase ELISA assay in CBS

ABL	EphA4	FGFR4	JAK1	SRC
ACK	EphA5	FGR	JAK2	SRM
ALK	EphA6	FLK1(KDR)	JAK3	SYK
ARG	EphA7	FLK2 (FLT3)	KIT	TEC
AXL	EphA8	FLT1	LCK	TEK(TIE2)
BLK	EphB1	FLT4	LTK	TNK
BMX	EphB2	FMS (CSFR)	Lyn	TRKA
BRK	EphB3	FRK	MER	TRKB
BTK	EphB4	FYN	MET	TRKC
CSK	FAK	HCK	PDGFRα	TXK
CTK	FER	HER2	PDGFRβ	TYK2
EGFR	FES	HER4	PYK2	TYRO10(DDR2)
EphA1	FGFR1	IGFIR	RET	TYRO3(Rse)
EphA2	FGFR2	INSR	RON	YES
EphA3	FGFR3	IRR	ROS	ZAP70

Currently 75 tyrosine kinase ELISA is availble from CBS. The rest of 3 (ITK, MUSK and TIE1) assays are available soon. For the tyrosine kinases in BLUE letters, IMAP^{TM} assays are also under development.

Conclusion

Carna Biosciences Inc., has developed 14 serine/threonine Kinase IMAP^{TM} assays. The Z'-factor of these assays were constantly more than 0.7 and this assures the robustness of the assays. Also, we have obtained the high reproducibility of IC_{50} values by Staurosporine which can prove the accuracy of our assays. IMAP^{TM} is superior homogeneous assay platform which is applicable to various kinase assays.

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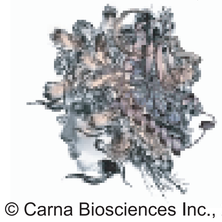
"Carna" is a goddess in Greek mythology who protects human health. This image is the symbol of Carna Biosciences Inc.,



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