Comprehensive Protein Kinase Profiling Panels for Inhibitor Selectivity Screening

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Abstract

It is well known that protein kinases are key elements in intracellular signaling pathways that control many physiological processes. Further, it has been demonstrated that the activity of protein kinases are altered in several human diseases such as cancer and autoimmune disorders. Therefore, molecules that modulate kinase functions are expected to be promising new drugs. To avoid predictable side effects of protein kinase inhibitors, determining their specificity is an important issue. Recently, Carna Biosciences have succeeded in extracting and cloning the entire (88) tyrosine kinase genes and is developing a complete tyrosine kinase assay panel for kinase inhibitor specificity profiling. A description of this panel system, as well as progress made with developing a profiling system for serine/threonine kinases will be presented. Each kinase assay will be available in homogeneous platforms using IMAP™ technology, TR-FRET and AlphaScreen™, all of which are suitable for HTS assays.

Tyrosine Kinases in Carna Biosciences

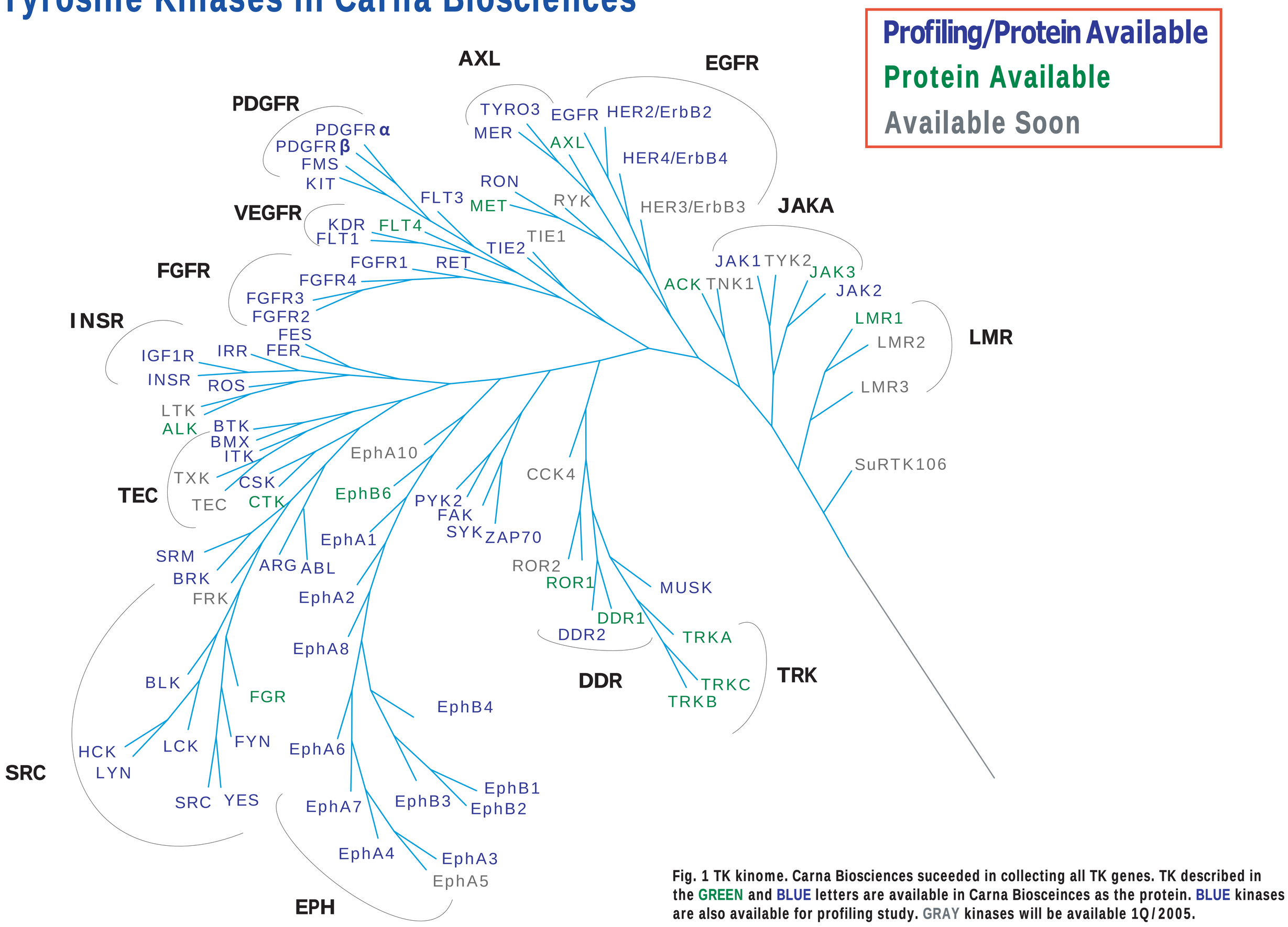


Fig. 1 TK kinome. Carna Biosciences suceeded in collecting all TK genes. TK described in the GREEN and BLUE letters are available in Carna Biosceinces as the protein. BLUE kinases are also available for profiling study. GRAY kinases will be available 1Q/2005.

Serine/Threonine Kinases in Carna Biosciences

AGC		CAMK		STE
AKT2	PKC-ε	CaMK4	PHKg1	MAP2K3
BARK1	PKC-γ	CaMK2-α	PIM1	MAP2K7
BARK2	PKC-θ	CHK1	PKD2	MAP3K5
CRIK	ROCK1	CHK2	TRIO	MAP3K3
PDK1	ROCK2	DAPK1		
PKA-cα	RSK2	MAPKAPK2		
PKC-α		smMLCK		

Profiling/Protein Available
Protein Available
Available Soon

Fig.2 STKs collecting in Carna Biosciences. We are also trying to clone entire serine / threonine kinase genes. The kinases described in the GREEN and BLUE letters are available in Carna Biosceinces as the protein. BLUE kinases are also available for profiling study. GRAY kinases will be available in 2005.

TK Profiling

Carna Biosciences is developing entire TK profiling panel firstly with 96-well format ELISA platform to exclude potential compound interference.

Procedure

Compound
↓
Substrate / ATP / Divalent Metal Ion
↓
Enzyme
↓
Wash, Blocking
↓
Conjugation
↓
Wash
↓
Enzyme Reaction for Detection
↓
Stop
↓
Measure

Fig. 3 Standard scheme of ELISA. Streptavidine coated-plate, biotinylated peptide substrates (which contains 1 tyrosine in their sequences) are used in the kinase reaction and HRP-conjugated anti-phospho antibody and TMB were used for detection. Absorbance at OD 450 nm was measured.

Km for ATP

Carna Biosciences's standard condition to evaluate inhibitory activity of compounds is performed with ATP at the concentrarions around Km. The most of known kinase inhibitors so far are the ATP competitor. Using too much ATP may miss the positive signal.

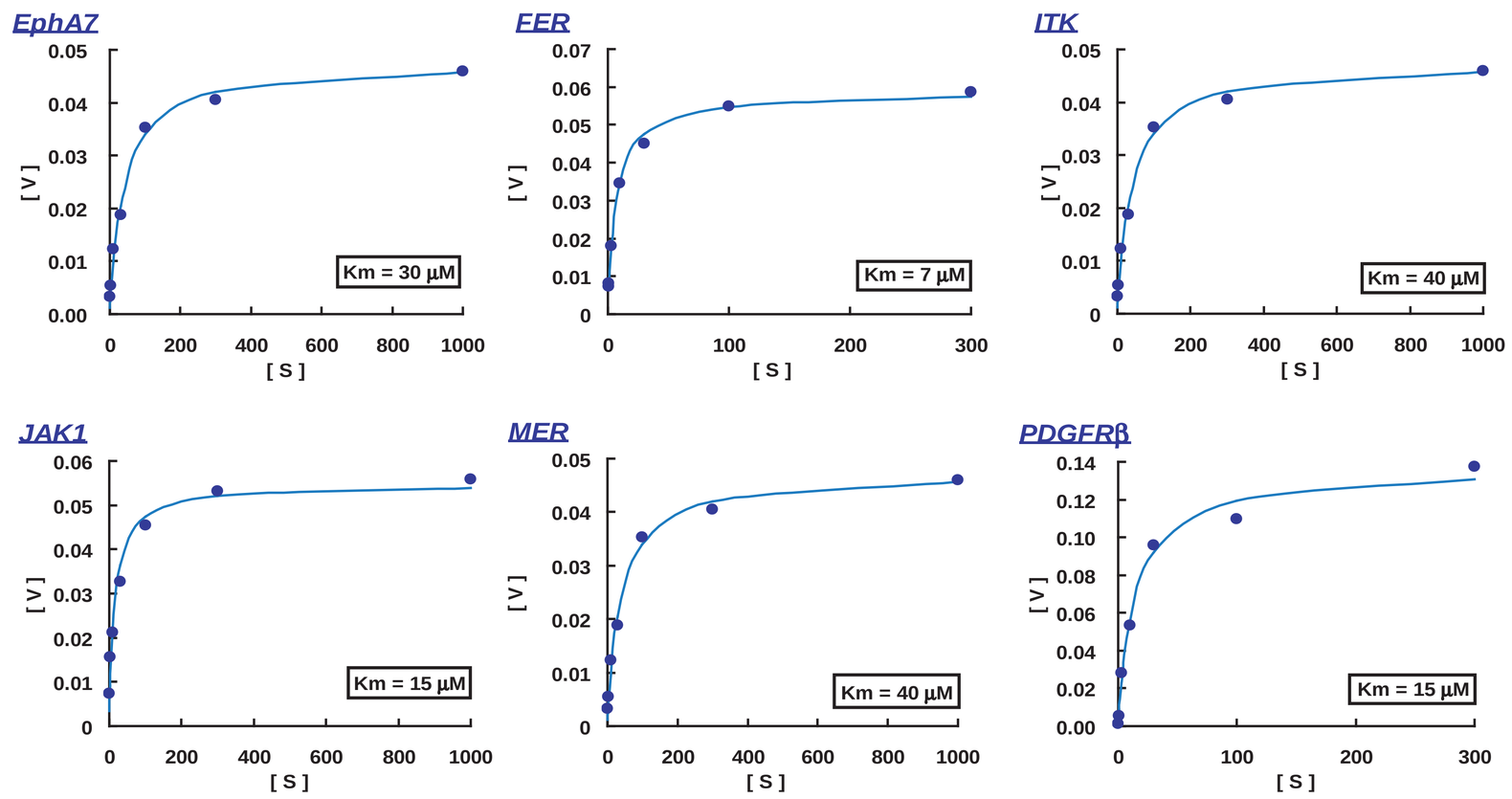


Fig.4 Representative data of Km for ATP. The Michaelis-Menten plot calculated from ATP concentrations and initial velocity values were shown. Initial velocity was determined by a linear least-squares fit from the data obtained with 5 to 30 min reaction. ATP concentrations were varied from 0.3 to 1000 nM by 3 in common ratio. The Km values for EphA7, FER, ITK, JAK1, MER and PDGFRβ were 30, 7, 40, 15, 40 and 15 µM, respectively. The values were 1 to 100 µM for the most of tested (62) TKs.

IC50 Determination (Staurosporine)

To validate the assay, IC50 for non-specific kinase inhibitor Staurosporine was determined.

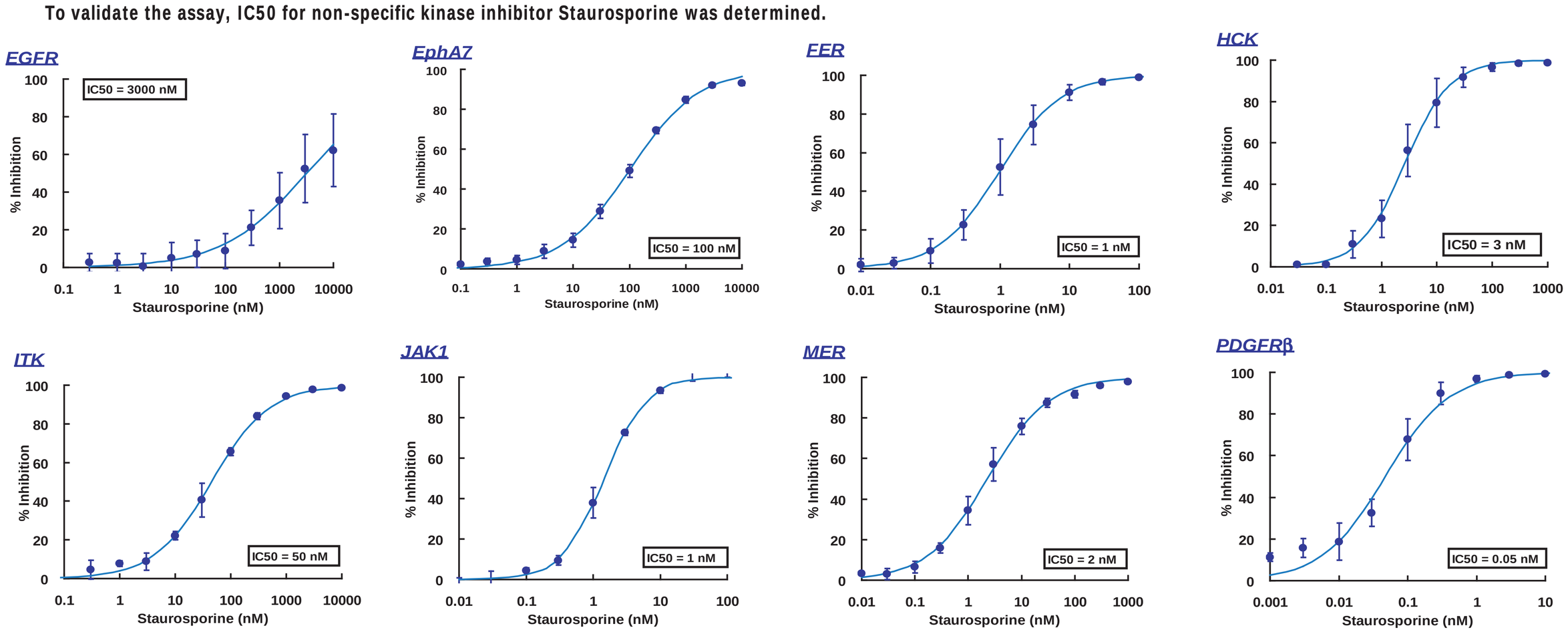


Fig.5 Representative data of IC50 values for Staurosporine. The ATP were used at the concentrations around Km for each kinase. The concentrations of each kinases were lower than 250 ng/mL. The IC50 values for EGFR, EphA7, FER, HCK, ITK, JAK1, MER and PDGFRβ were 3000, 100, 1, 3, 50, 1, 2 and 0.05 nM, respectively.

Tyrosine Kinase Profile (Staurosporine)

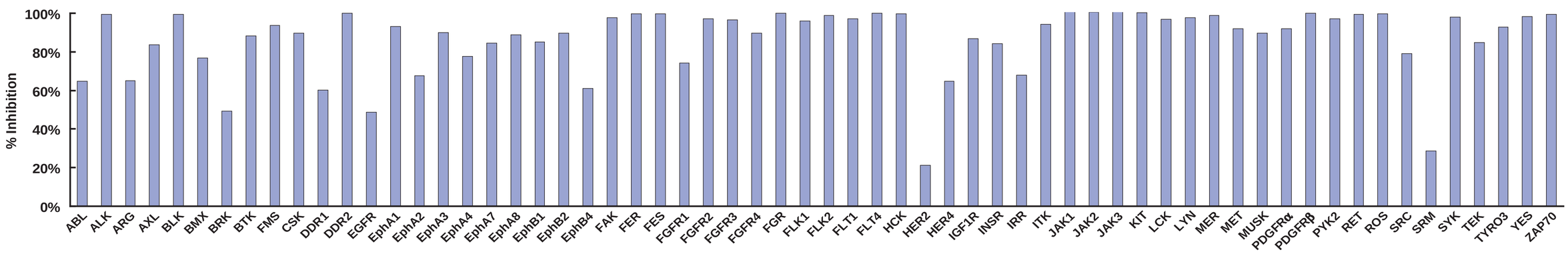


Fig.6 Inhibition study with 62 TKs were conducted with Staurosporine at 1 mM. The ATP were used at the concentrations around Km for each kinase. Staurosporine showed more than 50 % inhibition on the most of tested TKs.

IMAP™

The IMAP™ fluorescence polarization (FP) assay platform is a generic, homogeneous system applicable to a variety of enzymes, including protein kinases. IMAP™ is based on the high affinity binding of phosphate to immobilized trivalent metals. It has been applied to a wide variety of kinases spanning the whole kinome.

Principle

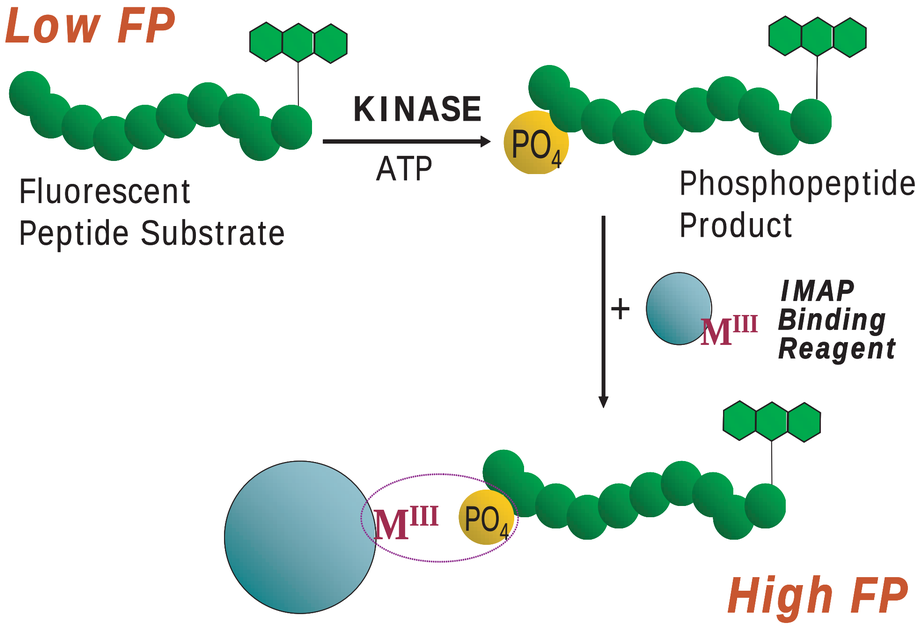
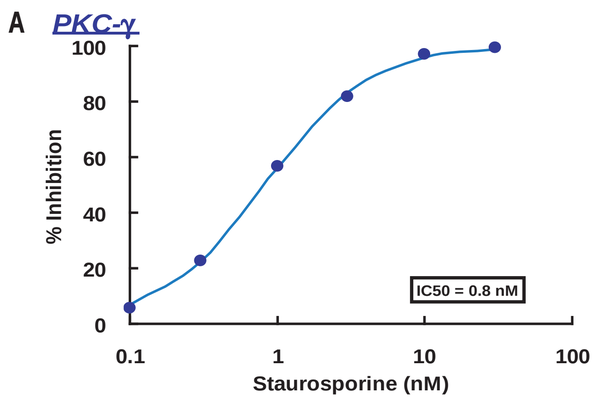


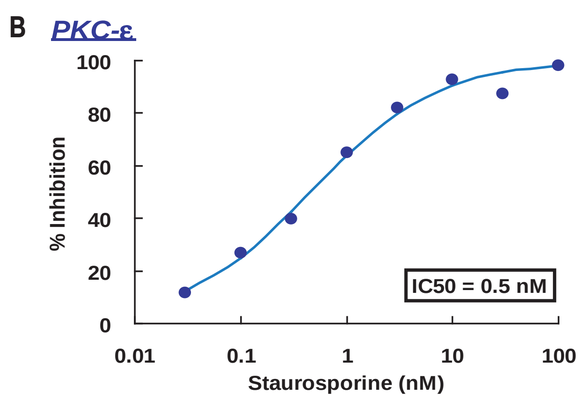
Fig.7 The IMAP™ technology is based on the high affinity binding of phosphate by immobilized metal (M^{III}) coordination complexes on nanoparticles. This IMAP™ "binding reagent" complexes with phosphate groups on phosphopeptides generated in a kinase reaction. Such binding causes a change in the rate 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, unlike antibody-based kinase assays, is applicable to a wide variety of kinases without regard to the substrate peptide sequences. IMAP™ can be also used to analyze phosphatase activity, simply by starting out with fluorescent phospho-peptide. This figure and the legend are by courtesy of Molecular Devices Corporation.

Serine / Threonine Kinase IMAP

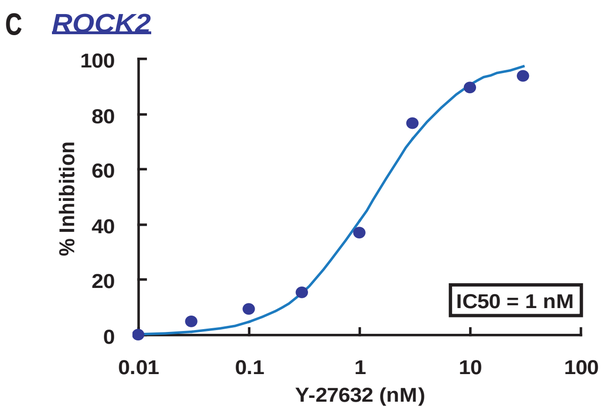
Carna Biosciences has been developing serine / threonine kinase IMAP™ assay. Currently, six serine / threonine kinases are available and the number is increasing rapidly.

Staurosporine





Y-27632



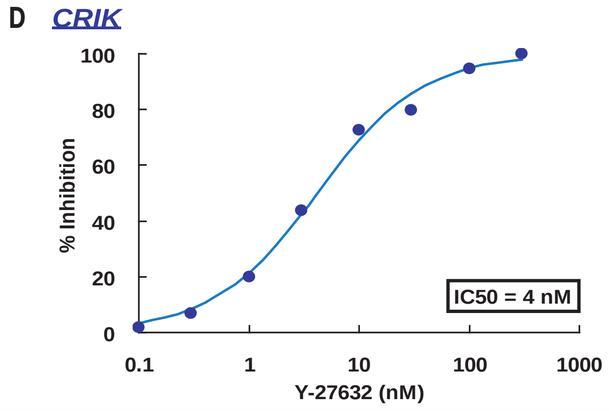
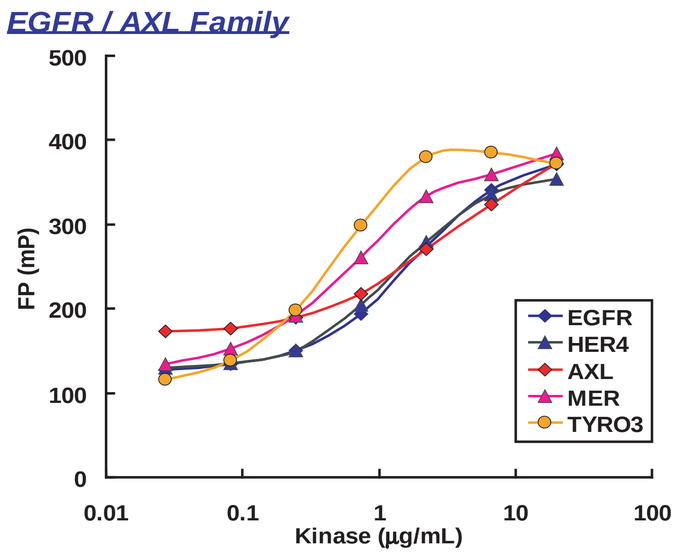
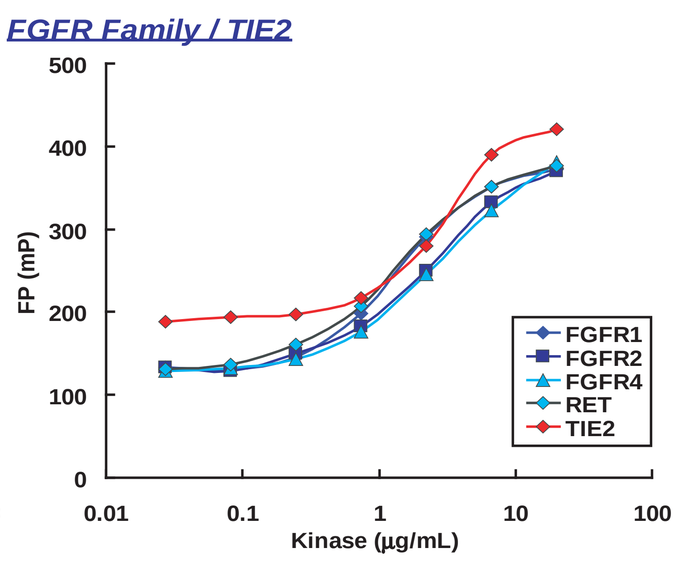


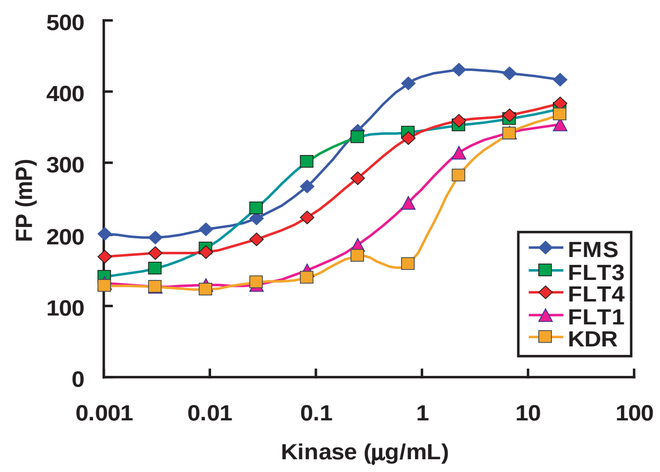
Fig.9 Representative data of IC50 values by Staurosporine A), B), or Y-27632 C), D). ATP was used at the concentrations around Km for each kinase. he IC50 values for PKC-γ, PKC-ε, ROCK2 and CRIK were 0.8, 0.5, 1 and 4 nM, respectively.

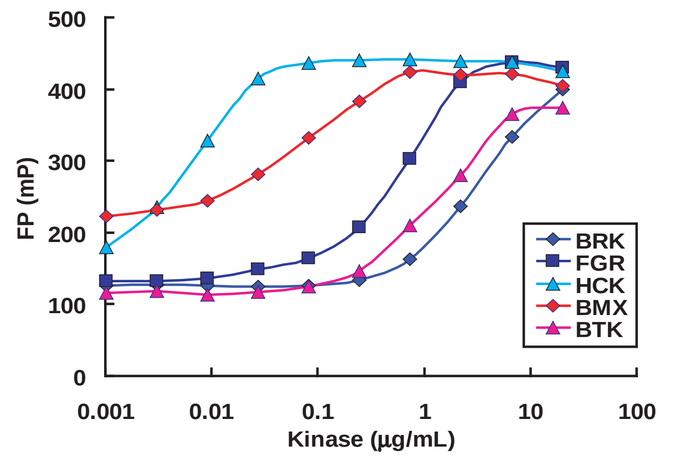
IMAP-able TKs

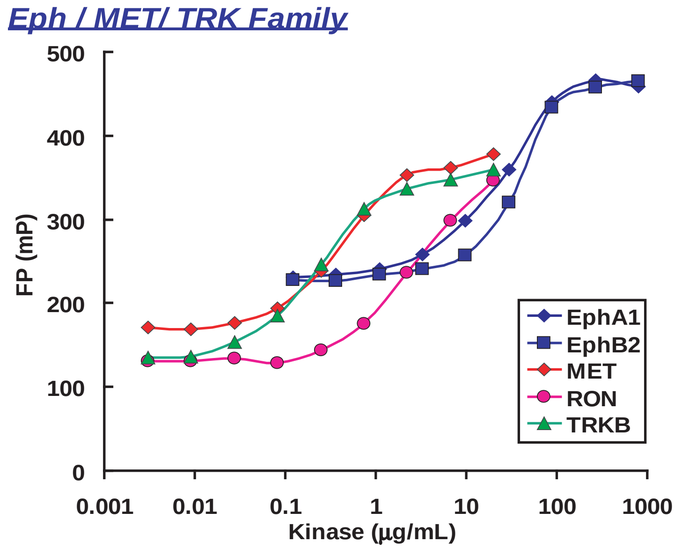
To apply the TKs to HTS assay, TK titrations were performed with IMAP™ platform.











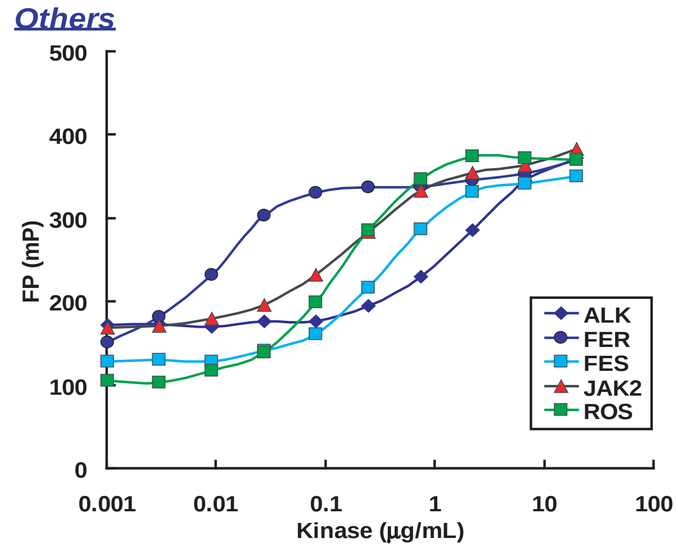


Fig.8 Representative data of TK IMAP™ assay. All the TKs currently available in Carna Biosciences were tested with IMAP™. Using 6 different substrates, 42 TKs were implied as IMAP™ applicable, the EC50 values were under 5 μg/mL. HCK showed lowest EC50 value at 7 ng/mL. This experiment was conducted in collaboration with Dr. Liz Gaudet, Molecular Devices Corporation.

Summary

Carna Biosciences has been creating entire TK profiling panel.
Currently 62 kinds of TK profiling panel has been completed.
Validation with Staurosporine proved the reliability of the assay.
These TKs were potentially applicable to IMAP™ homogeneous assay.
The rest of TKs for profiling and STK profiling will be available soon.

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