No.03
SUMMER 2010

Carna Biosciences, Inc. NEWSLETTER

Carna's Review

New Products & Services

Biotinylated *active* Kinases

Coming Soon!

Available at higher production levels, we can now support a wider range of assays such as Surface Plasmon Resonance (SPR), TR-FRET-binding assays, Protein Arrays, and many additional applications.

Protein Kinases

Cat# 02-166 **TSSK3**
Cat# 07-138 **TNIK**
Cat# 08-524 **FGFR4[N535K]**
Cat# 08-525 **FGFR4[V550E]**

Profiling

(Last Updated on May 10, 2010)

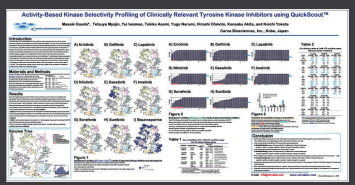
304 Kinases — ATP conc.= **Km app.** Profiling Service

153 Kinases — ATP conc.= **1mM** Profiling Service

Advertisement

Announcement

Our scientists presented posters at the SBS 16th Conference (April) and the CHI's 8th Next-Gen Kinase Inhibitors Conference (June) . Reprints are available at our website, www.carnabio.com.



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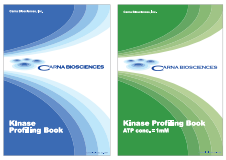
PROFILING

Profiling Services Updated

Our QuickScout Profiling Services have been updated, as of May 10th, to include PKAC γ (PRKAGC), bringing our current offering of assays performed at ATP Km app. to a total of 304.

Our optional **1mM ATP profiling service** was also extended by the addition of 56 Tyrosine kinases to a total of 153 (101 TKs including mutated forms, and 52 STKs). The use of these paired studies permits the rapid visualization, assessment, and determination of the impact ATP concentration exerts on kinase-inhibitory activity.

For more information, please download the **Kinase Profiling Book** from our website.



ASSAY KITS

Additional Ready-to-Run QSS Assist™ MSA Kits Released

Our original QSS Assist™ MSA kit (800dp) has been supplemented with a newly formatted 400dp kit, providing more speed, flexibility, and ease-of-use to our customers opting to perform Kinase Screening procedures in-house. These kits provide as many as 4 different kinases, with VALIDATED ASSAY PROTOCOLS, and quantities of essential reagents sufficient for aliquoting prior to use.

For additional information, please contact your sales representative.

Kinases in Press

Summarized & Commented by Carna R & D

A kinase research group at Brigham & Women's Hospital and Harvard Medical School recently published this article on haspin and DYRK2 inhibitor. They screened approx. 140,000 compounds for haspin activity using TR-FRET, and identified an acridine analog as a potent inhibitor. **Profiling against a panel of 270 kinases*** revealed that the compound also exhibited potent inhibitory activity for DYRK2, even these kinases are not structurally related Additional studies generated more specific inhibitors for haspin (180-fold selectivity over DYRK2) and for DYRK2 (5.4-fold selectivity over haspin). Further optimization of these inhibitors utilizing co-crystallization may provide even more potent inhibitors for both kinases.

* Determined by Carna Biosciences. The number of kinases in the profiling panel is currently 304 - the largest panel in the world for activity-based enzymatic analysis.

Reference: Bioorg. & Med. Chem. Letters, in press. (accepted manuscript is available online on 7 May 2010)

Scientist's Note from Production Team

Original Function of Kinase Observed in Production Process

Carna currently produces more than 330 active, recombinant human protein kinases, with all processes performed in-house. The majority of these products are expressed using the Sf21 cell line (isolated from ovarian tissue of the fall army worm, Spodoptera frugiperda), and the Baculovirus Expression Vector System (BEVS). Genes targeted for expression are inserted downstream of the polyhedron promoter, resulting in high-level expression of the desired recombinant kinase protein. In this article, we describe some of the interesting host-cell phenotypes generated in the production of kinases belonging to the MARK family and to TAK1-TAB1 kinases.

Kinase-induced morphological alterations to the host cell

Expression of MARK family kinases in Sf21 cells results in the appearance of protrusions and protuberances from the normally round-shaped cell surfaces (Picture), MARK kinases belong to the kinase family which phosphorylate the tau protein and ultimately, cause microtubule de-polymerization. Tau is a microtubule binding protein known to enhance tubulin polymerization and also to stabilize microtubules. When tau is hyper-phosphorylated, its binding potential to microtubules is reduced, resulting in unstable microtubules. In this process, neurofibrillary 'tangles' arise, which may lead to degeneration of the nervous system. For example, in Alzheimer's disease, some abnormalities are known to arise in tau. The morphological change we observed during the production of MARK family with Sf21 might be the result of this kinase exerting such an influence on the Sf21 cell cytoskeleton.


Normal Cell


MARK expressing cells

Kinase-induced size changes of the host cell

When TAK1 is expressed in Sf21 cells, we observe that the cell expands to a larger volume than normally visualized. An apoptotic response to such osmotic stress is a typical physiological action of TAK1 protein. TAK1 guides phosphorylation of JNK, a downstream kinase of the MAPK family via a MAP kinase cascade. Phosphorylated JNK promotes various gene expression in response to such stress. Expansion of Sf21 cell may be the result of afferent osmotic adjustment of the cell induced by elevated expression of active TAK1.

We constantly observe and record such phenotypic changes in host cells which may be related to the recombinant protein they express while in the manufacturing process. These and other observations have led to increased productivity in our cell cultivating processes, and better understanding of the physiological impacts of specific kinase hyper-expression in cultured cells.

Reference: Huangfu WC et al. J Biol Chem. 2006 Sep 29; 281(39):28802-10.

We have optimized our cell culture processes with the specific goals of scale-up and specific kinase yield improvement, incorporating new equipment as necessary. As a result, some kinases previously categorized as 'low yield' have now become available in bulk. Please contact us if you experience troubles or concerns with production yield, purity, etc. We will be pleased to assist your drug discovery research with various tools.