



INTRODUCTION

Due to the critical roles in intracellular communication, dysregulation of protein kinases has been implicated in as many as 400 human diseases including cancer, diabetes, heart diseases, neurological disorders and rheumatoid arthritis. Hence, individual protein kinases are important for drug target validation, protein crystal structure analysis, and drug design and screening. Promising drug leads can be screened against a panel of kinases, enabling scientists to determine the selectivity of new chemical entities. Regardless of the purpose, enzymes need to be active, highly purified, and ideally, reflect the natural post-translational modifications of the physiologically authentic protein (native state of human proteins in human cells). Currently, kinases are predominantly produced in non-human cells (e.g. E coli or insect cells) many of which involve protein truncation and/or in vitro activation, due to the limitation of the expression system.

HumanZyme has developed an efficient human cell-based technology, HumaXpress™, for the rapid production of active recombinant human protein kinases which are full length, *in vivo* activated, and highly authentic. MAPKAP kinase2 (MK2) is a Ser/Thr protein kinase, which is regulated through direct phosphorylation by p38 MAP kinase. In conjunction with p38 MAP kinase, this kinase is known to be involved in many cellular processes including stress and inflammatory responses, nuclear export, gene expression regulation and cell proliferation. In this study, we demonstrate that the authentic MK2 from human cells is differentiated from the non-human cell version in a way that will greatly improve the speed and quality of both basic research and pharmaceutical development.

MATERIALS AND METHODS

MK2- The HumanZyme MK2 kinase is full-length containing a N-terminal GST tag. The protein was produced and activated *in vivo* (in human cells) in the presence of arsenite. MK2 kinase from Vendor A is a truncated form containing residues 46-400 and an N-terminal GST tag. The protein was expressed and purified from E. coli, in vitro activated with MAPK2, and repurified on Q-Sepharose FF.

Kinase Assay- The kinase assay used at Amphora Discovery detects the direct phosphorylation of a fluorescently labeled peptide substrate analog. Using Caliper's microfluidic technology, phosphorylated product can be separated from substrate providing a quantitative determination of the ratio of the phosphorylated peptide pool.

Reaction Conditions- Unless otherwise noted these are the conditions for the MK2 kinase assay for 3 hr incubation at room temperature (25 °C):

100 mM HEPES, pH 7.5; 1 mg/ml bovine serum albumin; 0.01% Triton X-100; 1 mM DTT; 10 mM MgCl₂; 10 mM β-glycerophosphate; 10 mM sodium orthovanadate; 1 μM FAM-KKLRRTLSVA-CONH₂ (fluorescently labeled peptide); 100 μM ATP; 1% DMSO.

Peptide Identification- HumanZyme and Vendor A MK2 were screened for kinase activity against a collection of 192 kinase substrate peptides using the conditions described above but with 100 μM ATP

RESULTS

Purity and activity of HumanZyme MK2- SDS PAGE analysis shows that the enzyme is pure with a dominant band of 62 kD and minor band of endogenous human GST of 23 kD. The specific activity was 184 U/mg. One unit of kinase activity is defined as 1 nmole ATP consumed in the kinase reaction assay in the presence of 30 μ M substrate peptide KKLNRRLSVA per minute at 30°C with a final ATP concentration of 80 μ M.

Substrate identification- Two peptides were identified as a substrate for Humanzyme MK2, KKLRRRLSVA and KIRRLSVA. These substrates were also identified as a substrate for the Vendor A MK2. The KKLRRRLSVA sequence was used to determine the biochemical parameters of the assay

K_m Determination- The ATP K_m for the kinase reaction using the identified peptide was determined using the above reaction conditions but with varying ATP concentrations. The ATP K_m for the HumanZyme preparation is 5 ± 0.8 mM while the K_m was 15 ± 2.2 mM for the Vendor A preparation (Fig. 1).

Inhibitor IC₅₀- The IC₅₀ values were determined for 13 known kinase inhibitors (Fig. 2). Reactions were as described above and incubated for 3 hr at room temperature. There was a difference in the sensitivity of select set inhibitors between the two preparations (Table 2). Both preparations were sensitive to staurosporine, AMP-PNP (a non-hydrolysable ATP analog), and K252a. The Vendor A MK2 was more sensitive to staurosporine and K252a with IC₅₀ values 30-70 fold less than the Humanzyme MK2. In contrast, the Humanzyme MK2 was more sensitive to AMP-PNP than the Vendor A preparation, which may be a reflection of the differences in the ATP K_m.

Figure 1. ATP K_m

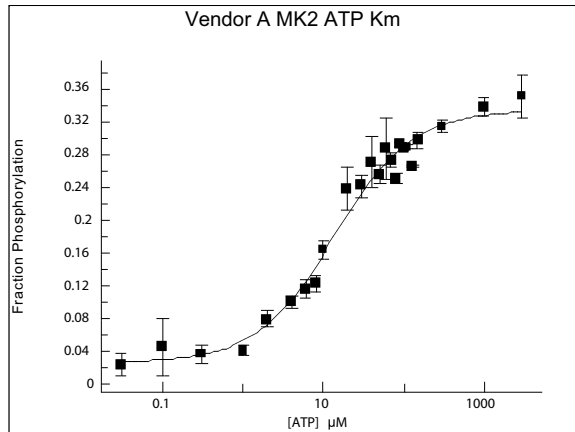
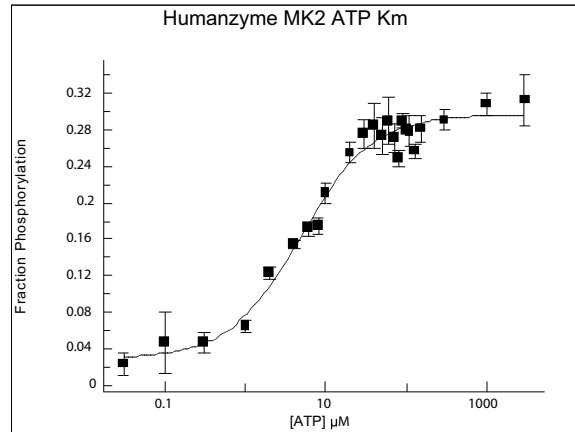


Table 1. IC₅₀ Values

	HumanZyme IC ₅₀ μ M	Vendor A IC ₅₀ μ M
Staurosporine	10	0.3
H-9 dihydrochloride	>10	>10
AMP-PNP	872	2000
HA-1077 dihydrochloride	>10	>10
Rottlerin	>200	>200
H89 Dihydrochloride	>10	>10
5-iodotubercidin	>10	>10
K252a	3.5	0.05
Ro 32-0432	>10	>10
Ro 31-8220	>10	>10
GF 109203X	>10	>10
KT5720	>10	>10
Imatinibmesylate	>10	>10

CONCLUSION

The current study demonstrates that the properties of the human protein kinase MK2 produced in human cells are distinct from the non-human cell version. Given the high cost and lengthy development of drugs, it would be highly justified to use human kinases with high authenticity for drug screening as well as kinase profiling. This will allow researchers to avoid pursuing false negative leads and missing promising targets.

This study was conducted in collaboration with Amphora (amphoracorp.com)

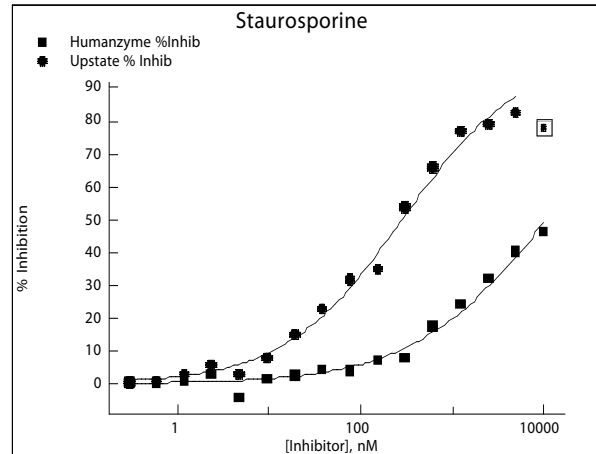
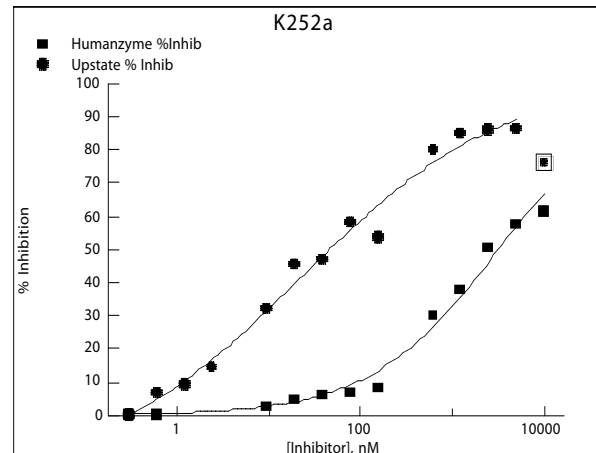


Figure 2. Inhibition Curves



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