Custom-tailored Development of Kinase Activation Assays

REACTION BIOLOGY

TARGET-SPECIFIC ASSAY SERVICES

➢ Introduction

Protein kinases are important therapeutic targets and many protein kinase inhibitors have successfully been developed for the treatment of various cancers.
Most of these compounds exert their kinase inhibiting function by competing with ATP either by binding to the ATP-binding pocket directly or to conserved structures in close proximity.

Due to the similarity in the structures of catalytic domains of active kinases the development of target selective compounds has been a challenge. Enhanced target selectivity, however, could be achieved by inhibitors that target allosteric sites apart from the core kinase domain.
The identification of such inhibitors requires an experimental setup in which the kinase is converted from its non-active to its active conformation by physiological upstream activators in the absence or presence of the test compounds. Such assays may even address the effect of compounds on a complete signal transduction cascade including two or more upstream-downstream kinase activation pairs.

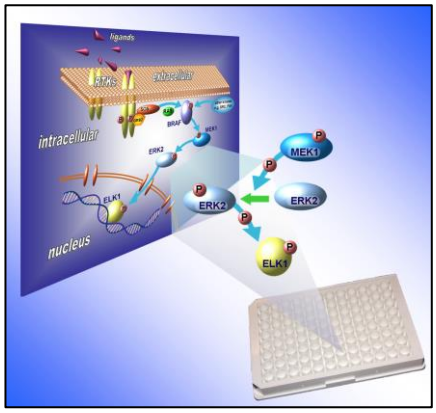


Figure 1: Schematic outline of MEK1-ERK2 activation cascade.

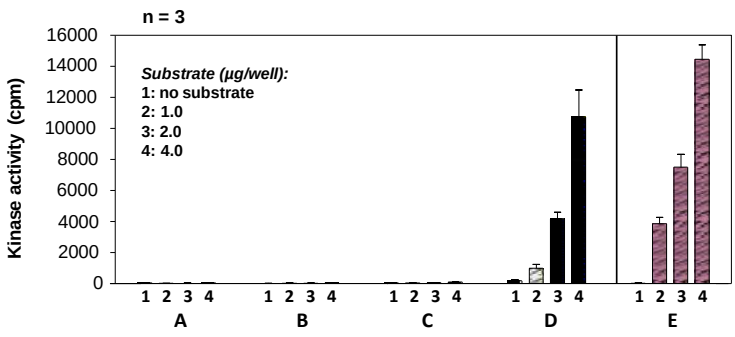
➢ Field of application

Kinase Activation Assays developed by Reaction Biology can be used e.g. for high-throughput screening campaigns performed to identify compounds which are able to interfere with the activation of downstream kinases by their respective upstream kinases. The assays may analyse single step auto-activation as well as multistep trans-activation ("activation cascades").

➢ Case study: Kinase Activation Assay MEK1-ERK2

MEK1 dependent activation of ERK2 was arbitrarily selected as a case study example. ERK2 was activated using MEK1 (activated by BRAF) as the upstream activating kinase in a 1:10 ratio. A >20fold increase in ERK2 dependent substrate phosphorylation could be achieved, while MEK1 phosphorylation of the substrate was not detectable (figure 2).
In a case study using a library of >1500 kinase focused compounds a hit rate of 10.1% could be achieved using this experimental setup. Several inhibitors of the MEK1-ERK2 Kinase Activation Assay were further analysed by comparing their inhibitory activity against the cascade with their effect on the individual kinase/substrate pairs (MEK1(active)-ERK2 K54R; ERK2(active)-RBER-CHKtide). 7.5% of the compounds showed an inhibitory effect in the activation assay but not in the individual assays, indicating a potential allosteric mode of action e.g. by interfering with binding of MEK1 to unactivated ERK2 or masking of activatory phosphorylation sites.

Figure 2: Flashplate® based ERK2 activation assay.
Non-activated ERK2 was incubated with active MEK1 (D) in the presence of various amounts of a generic ERK2 substrate (RBER- CHKtide) and 5 µM ATP. Neither non-activated ERK2 (C) nor active MEK1(B) on their own could phosphorylate RBER-CHKtide above substrate background signal (A). Activation of non-active ERK2 by catalytic amounts of active MEK1 resulted in ERK2 activity comparable to that of pre-activated ERK2 (compare D and E).



➢ Available service by Reaction Biology for Kinase Activation Assays

Based on our extensive know-how and vast reagent collection for establishing standard in vitro kinase activity assays for more than 380 kinases, Reaction Biology offers to establish single or multistep in vitro Kinase Activation Assays according to customers' requirements. Our scientists will formulate a specific project proposal in close cooperation with the customer.
The assay establishment may include customized recombinant protein expression and/or modification of either kinases or substrates to generate an in vitro Kinase Activation Assay which will be able to provide relevant data to the customers' specific scientific problem in question.

➢ Available tools for custom tailored development of Kinase Activation Assays

Reaction Biology has recombinantly expressed and purified a number of other MAPK cascade's key elements and prepared them in defined conditions with regard to their respective activation status. These proteins may now be directly utilized for in vitro Kinase Activation Assays in a very similar way as shown for MEK1-ERK2. They are also available as recombinant proteins in µg to mg amounts.
A large number of other protein kinases, like receptor- and non-receptor tyrosine kinases as well as numerous additional serine-threonine kinases apart from MAPKs also require activating phosphorylation steps. Based on the assay technology used for the case study shown above, the successful establishment of in vitro Kinase Activation Assays for high-throughput compound screening should be feasible for a large number of these kinases.