

ZeptoMARK Reverse Arrays

Multiplexed Compound Selectivity Profiling

The High Content Approach

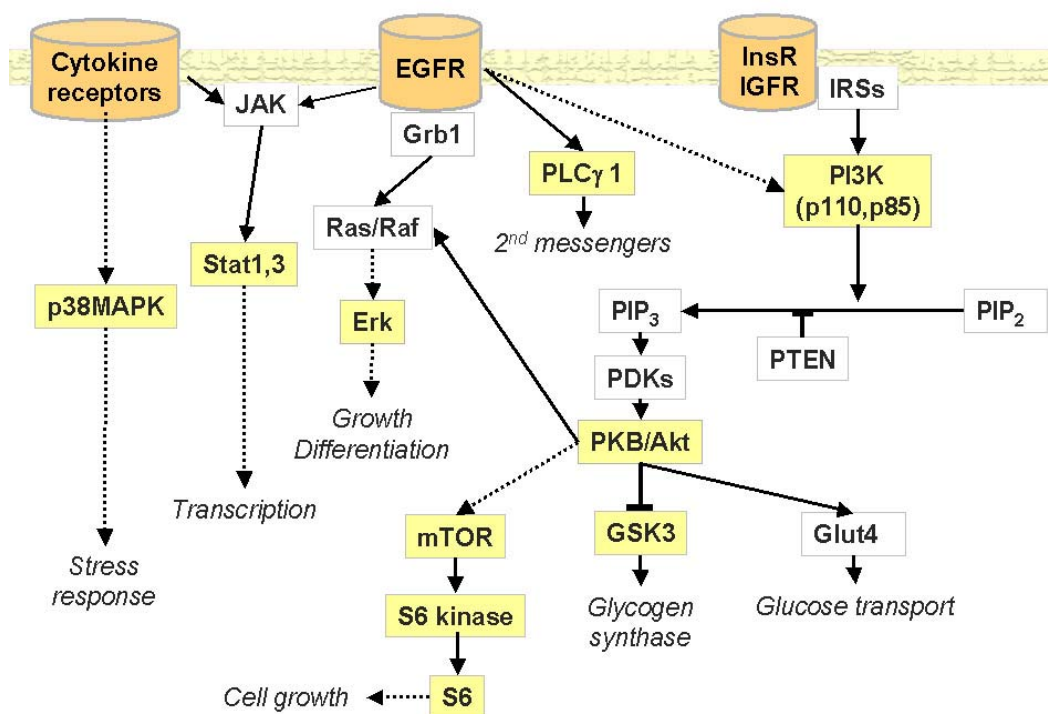
ZeptoMARK Reverse Arrays are the leading technology to screen compound efficacy at a cellular level for multiple pathways and downstream targets in a flexible and cost effective manner. Besides the targets of interest, corresponding stress and apoptosis pathway markers can be measured simultaneously, giving an indication not only of compound efficacy but also toxicity.

The reverse array format allows rapid implementation of site-specific phosphorylation assays, since in contrast to ELISA type systems only a single antigen specific antibody is required. The principle of reverse arrays is based on totally denatured samples, which makes it similar to a western blot analysis. Hence, a large commercially available base of antibodies is available for use already.

Tracing Pathway Activities with Kinase Inhibitors – The Experiment

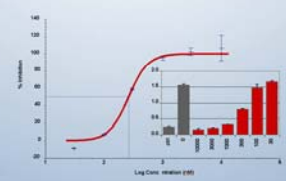
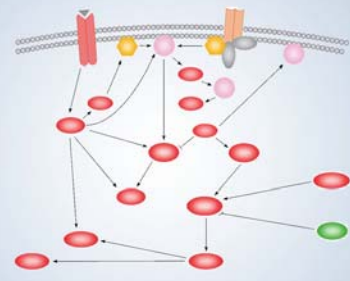
Cells were analyzed for the activation state of key effectors in the EGF and insulin signaling pathways to validate this model for compound screening. A standard microplate format for growing, treating and lysing cells was shown to be suitable for this approach, establishing the value of the technology against a wide variety of cellular signaling pathways.

A431 cells were grown and incubated with serial dilutions of 12 kinase inhibitor compounds followed by stimulation with EGF and lysis of the cells. Compound profiles were generated by measuring the phosphorylation



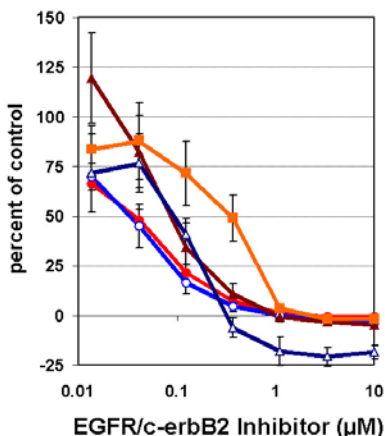
status of 5 different downstream effectors of the EGF pathway: EGFR, ERK, STAT3, PLCγ1 and AKT. The potencies of the 12 compounds for inhibition of EGF-induced protein phosphorylation were determined from dose response curves and quantified as transduced EC₅₀ values (trEC₅₀). trEC₅₀ values are a valuable measure for the distance between the original point of inhibition and the effects on down-

stream nodes in the signaling pathway. It also indicates dilution of the signal due to pathway branching and/or cross-talk.



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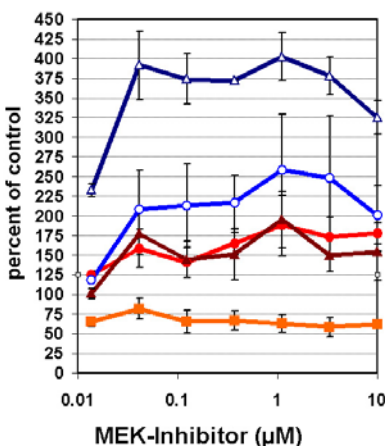
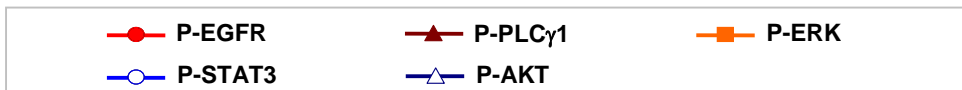
Three Outstanding Examples of Kinase Inhibition Profiles



Detection of Unexpected Inhibition Activity

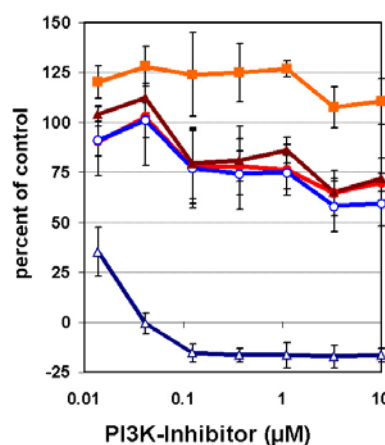
As expected, the EGFR/c-erbB2 kinase inhibitor included in this study, inhibited the ligand induced EGFR phosphorylation with different potencies. Consequently, phosphorylation of the downstream target proteins STAT3, PLC γ 1 as well as P-AKT was also reduced with $trEC_{50}$ values close to those for the EGFR.

For inhibition of ERK1/2 phosphorylation, however significantly higher $trEC_{50}$ values were obtained.



Determination of Apparent Lack of Efficacy

An indication for an atypical regulation of the MEK-ERK pathway in A431 cells is observed after treatment with a highly potent MEK-inhibitor. No dose response, but a plateau around 30-50% inhibition was obtained with this compound between 0.01 and 10 μ M. Another unexpected effect observed in MEK inhibitor treated A431 cells was the strong increase in AKT phosphorylation after stimulation with EGF; AKT phosphorylation was not expected to be affected by a MEK-ERK pathway inhibitor but may be a consequence of the increase in phosphorylation observed for EGFR. In addition, PCL γ 1 and STAT3-phosphorylation was also increased.



An Example of a Selective Inhibitor

The pan-PI3K inhibitor reduced only the phosphorylation of AKT, as expected. This compound turned out to be a highly selective and potent inhibitor.

Zeptosens is a full solution supplier of reverse array systems in compound screening and systems biology research.