Cellular Phosphorylation Assay for TIE2

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

➢ The Target

The angiopoietin (Ang)-Tie ligand-receptor system has a key role in regulating vascular integrity and quiescence. Besides its role in angiogenesis it is an important regulator in numerous diseases including inflammation. Ang-1-mediated Tie2 activation is required to maintain the quiescent resting state of the endothelium. In contrast, Ang-2 destabilizes the quiescent endothelium and primes it to respond to exogenous stimuli, thereby facilitating the activities of inflammatory and angiogenic cytokines. Intriguingly, Ang-2 is expressed weakly by the resting endothelium but becomes strongly upregulated following endothelial activation. Moreover, endothelial cells store Ang-2 in Weibel-Palade bodies from where it can be made available quickly following stimulation, suggesting a role of Ang-2 in controlling rapid vascular adaptive processes. This suggests that Ang-2 functions as a built-in switch controlling the transition of the resting quiescent endothelium towards the activated responsive endothelium (Trends Immunol. 2006 27, p552-8).

➢ Cellular Phosphorylation Assay

CHO cells have been stably transfected to overexpress full length human TIE2. Stimulation of these cells with sodium orthovanadate results in a robust receptor autophosphorylation. Compounds are preincubated before cell stimulation to allow thorough target binding. Stimulation conditions are optimized to determine dose-related inhibition of the phospho-TIE2 signal, which is subsequently quantified by Sandwich-ELISA technique. The assay is validated based on known inhibitors of TIE2 kinase activity (see Fig. 1).

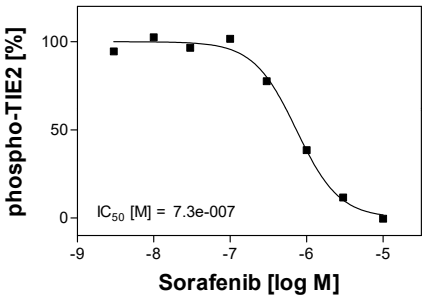
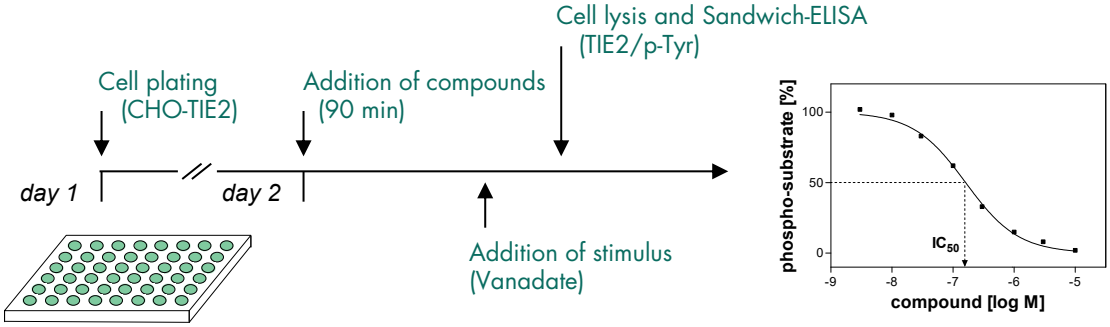


Figure 1: Assay validation.
Sorafenib is a potent inhibitor of TIE2 phosphorylation with highly reproducible IC_{50} values. The graph shows a representative result.

➢ You ship your compounds – Reaction Biology performs the testing



- IC_{50} values are determined by testing 8 compound concentrations in semi-logarithmic steps (each concentration in duplicates).
- Quality assurance is provided by calculation of Z' factors for Low/High controls on each assay plate and by including a full IC_{50} curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.