Cellular Phosphorylation Assay for AXL

CELLULAR ASSAY SERVICES

➢ The Target

AXL is a receptor tyrosine kinase with a unique structure of the extracellular region that juxtaposes IgL and FNIII repeats. AXL stimulation has been described to drive cellular proliferation and the protein growth arrest-specific gene-6 (GAS6) has been found to be a ligand. Dysregulation of AXL and its ligand GAS6 is implicated in the pathogenesis of several human cancers. Recently, it has been shown by siRNA knock-down that AXL plays a role in tumor growth, metastasis and angiogenesis (Li, Y et al. (2009) *Oncogene*).

➢ Cellular Phosphorylation Assay

Reaction Biology's cellular AXL phosphorylation assay was generated on a mouse embryonal fibroblast (MEF) background. Cells were transfected to express a full-length AXL protein. After clonal selection a transformed cell line with a high level of autophosphorylated AXL was obtained. By adding Sunitinib phospho-AXL levels are largely decreased and thus the dynamic behaviour to determine inhibitory potentials of compounds was achieved. Phospho-AXL levels are quantified by Sandwich-ELISA technique.

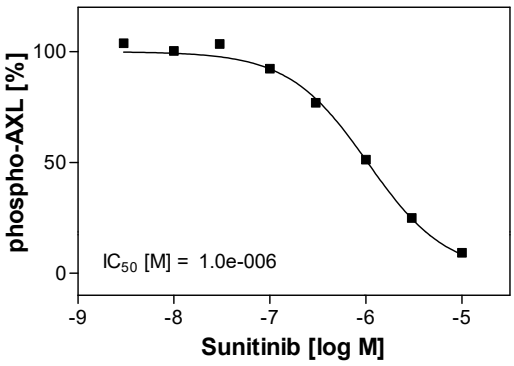
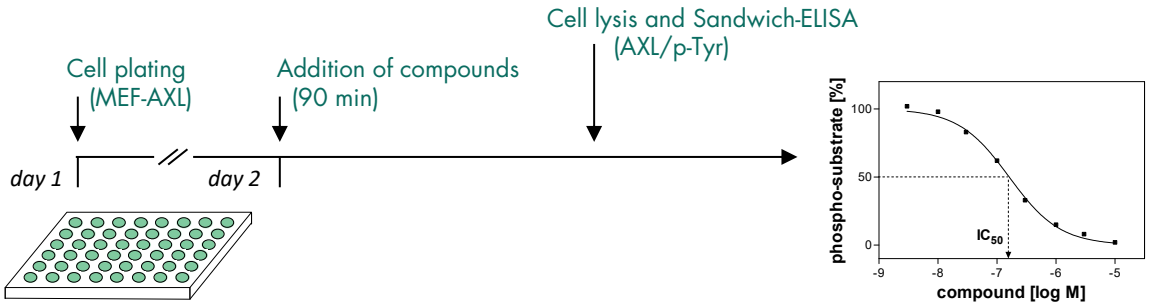


Figure 1: Assay validation.
Sunitinib is a potent inhibitor of the phospho-AXL signal found in the described cells. The graph shows a representative result.

➢ 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.