

➤ The Target

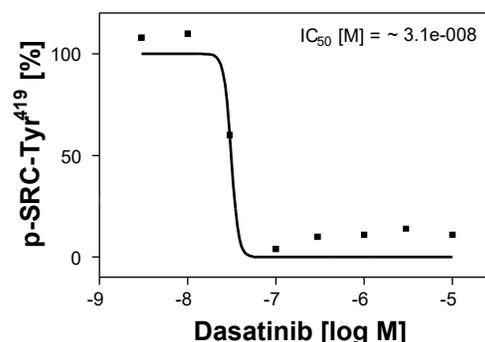
SRC is a 60 kDa protein consisting of several domains which mediate its diverse functions. The cytosolic SRC protein-tyrosine kinase is a regulatory protein that plays a key role in cell differentiation, motility, proliferation and survival. Activated Src is common in human tumors. Human SRC is rarely mutated into an oncogene but is rather dysregulated via overexpression, reduced degradation or mRNA stability. SRC activity has an important oncogenic role transmitting signals from other oncogenes such as EGFR, HER2, and PDGFR. In this context SRC is considered to promote the metastatic phenotype.

➤ Cellular Phosphorylation Assay

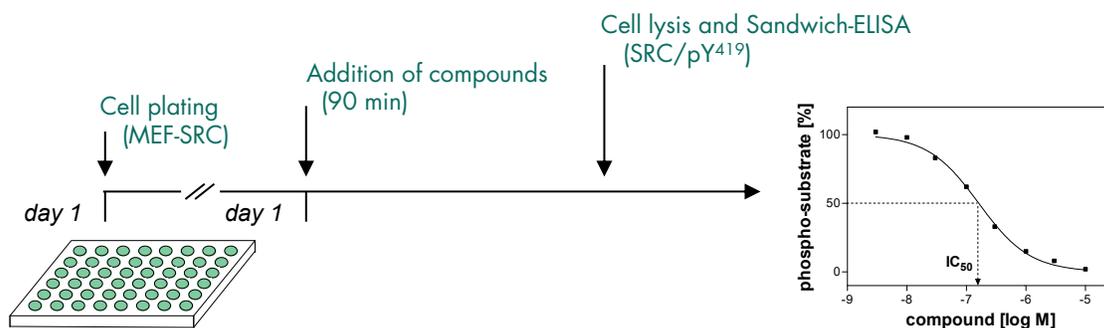
Reaction Biology's cellular SRC phosphorylation assay was generated on a mouse embryonal fibroblast (MEF) background. Cells were transfected to express a full-length SRC protein. After clonal selection a transformed cell line with a high level of autophosphorylated SRC was obtained. By adding Dasatinib phospho-SRC levels are largely decreased and thus the dynamic behaviour to determine inhibitory potentials of compounds was achieved. The assay quantifies activity of SRC by detecting phosphorylation of SRC autophosphorylation site Y419 using a self-developed solid-phase-ELISA.

Figure 1: Assay validation.

Dasatinib is known to inhibit the phospho-SRC signal in a highly specific manner. The graph shows a representative result.



➤ You ship your compounds – Reaction Biology performs the testing



- IC₅₀ 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 IC₅₀ curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.