Cellular Phosphorylation Assay for FYN

➤ The Target

FYN is a cytoplasmic non-receptor tyrosine protein kinase of Src family kinases (SFKs) implicated as a therapeutic target in cancer and neurological disorders such as Alzheimer's. FYN participates in several signaling interactions through its SH2/SH3 domains and plays a significant role in multiple biological processes e.g. growth factor/cytokine receptor signaling, ion channel function, platelet activation, T-cell and B-cell receptor signaling, axon guidance, fertilization, entry into mitosis, differentiation of natural killer cells, etc1. Additionally, FYN is important in brain development, peripheral immunity and is also known to mediate integrin adhesion as well as cell-cell interactions1. FYN activation depends on binding of ligands to the SH2/SH3 domains and on phosphorylation/dephosphorylation of two critical tyrosine residues, Y530 and Y419.

[1]Curr Med Chem. 2011;18(19):2921-42

## ➤ Cellular Phosphorylation Assay

In the cellular FYN phosphorylation assay the rat fibroblast cell line Rat1 is used, expressing exogenously introduced point mutant FYN-Y530F which is myc-tagged at the C-terminus. The Y530F point mutation results in constitutive kinase activation and autophosphorylation at Y419. Quantification of FYN phosphorylation is assessed via Sandwich ELISA using a substrate specific capture antibody and a phospho-Y419 specific detection antibody.

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

IC_{50} [M] = 2.5e-009
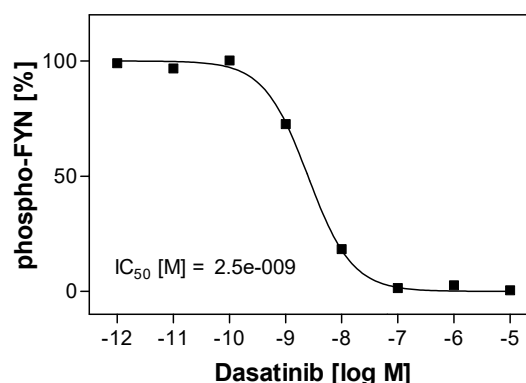
➤ You ship your compounds – Reaction Biology performs the testing
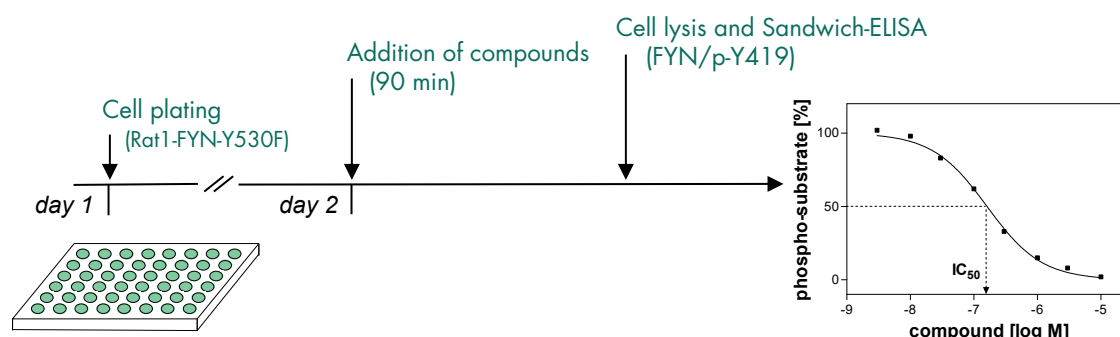
- IC_{50} values are determined by testing 8 compound concentrations in semi-logarithmic steps (each concentration in duplicates).
- Quality assurance is provided by calculation of Z' factors for Low/High controls on each assay plate and by including a full IC_{50} curve for a reference inhibitor to monitor adequate dose/response relation in your assay run.