Cellular Phosphorylation Assay for FYN

ProQinase TARGETING CANCER

Cellular Assay Service

➢ The Target

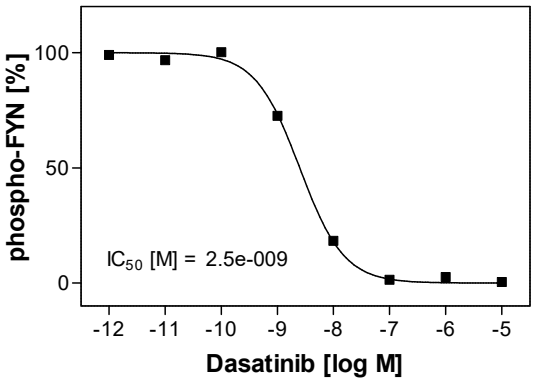
FYN is a cytoplasmic non-receptor tyrosine protein kinase of Src family kinases (SFKs) implicated as a therapeutic target in cancer and neurological disorders such as Alzheimer's. FYN participates in several signaling interactions through its SH2/SH3 domains and plays a significant role in multiple biological 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, etc[1]. Additionally, FYN is important in brain development, peripheral immunity and is also known to mediate integrin adhesion as well as cell-cell interactions[1]. 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

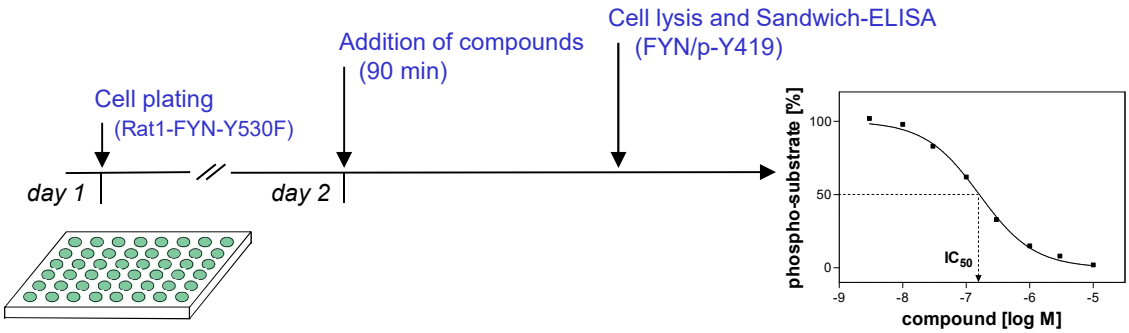
➢ Cellular Phosphorylation Assay

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.



➢ You ship your compounds – ProQinase 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.