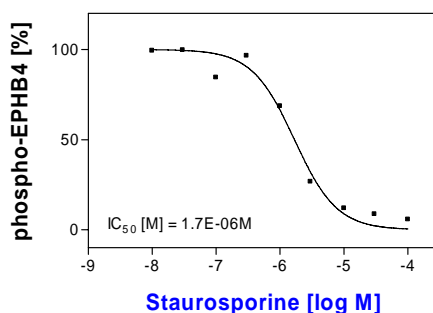


## The target

The receptor tyrosine kinase EPHB4 belongs to the family of Ephrin receptors. It is activated by binding to its membrane-bound ligand Ephrin-B2. Receptor and ligand are reciprocally expressed in venous and arterial endothelial cells and play an important role in angiogenesis. In several human tumors increased levels of the EPHB4 protein were identified, a finding that characterizes the receptor as a potential cancer target.

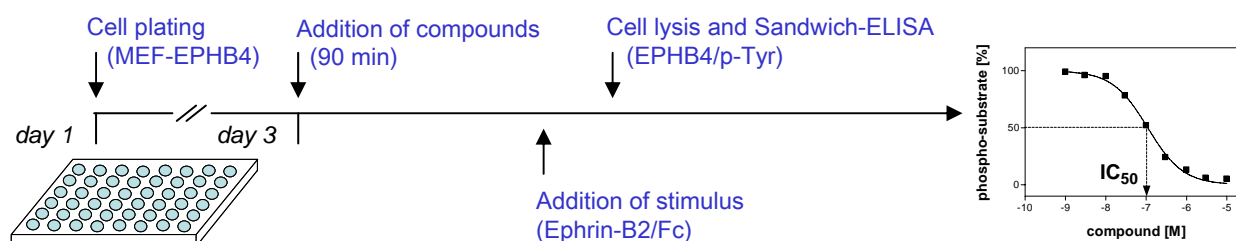
## Cellular phosphorylation assay



**Figure 1:** Assay validation. Staurosporine is a potent inhibitor of the Ephrin-B2 induced phospho-EPHB4 signal found in the described cells. The graph side shows a representative result of one experiment with Staurosporine.

ProQinase's cellular EPHB4 phosphorylation assay was generated on a mouse embryonal fibroblast (MEF) background. Cells exogenously express a full-length human EPHB4 molecule. Binding of a soluble Ephrin-B2/Fc Chimera activates receptor autophosphorylation via the mechanism of receptor oligomerisation. After stimulation with the ligand the phospho-EPHB4 levels are quantified by Sandwich-ELISA technique. The assay is validated based on known inhibitors of EPHB4 kinase activity (see Figure 1).

## You ship your compounds – ProQinase performs the testing



- IC<sub>50</sub> values are determined by testing 9 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<sub>50</sub> curve for Staurosporine to monitor adequate dose/response relation in your assay run.

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