Cellular Phosphorylation Assay for FGF-R2

ProQinase TARGETING CANCER

Cellular Assay Service

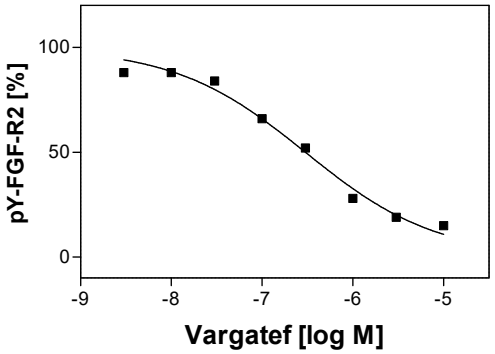
➢ The Target

Fibroblast growth factor receptor 2 (FGF-R2) is a member of the FGFR receptor tyrosine kinase family, which consists of 4 receptors and 23 ligands. Ligand binding leads to FGF-R2 dimerization, autophosphorylation and activation of signaling components including Akt and Erk kinases. FGF-R2 is amplified in 3-10% of primary gastric cancer, resulting in ligand independent receptor activity and cell transformation.

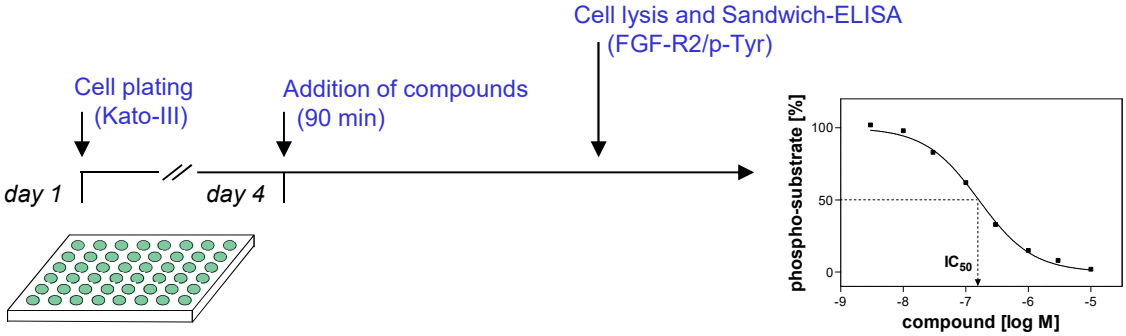
➢ Cellular Phosphorylation Assay

In the human gastric carcinoma cell line Kato-III, DNA-amplification results in overexpression of FGF-R2 which is associated with constitutive, ligand-independent autophosphorylation of this receptor. FGF-R2 activity is potently inhibited in the presence of cognate FGF-R2 inhibitors such as Vargatef (BIBF1120) (see Fig. 1). Phospho-FGF-R2 levels are quantified by Sandwich-ELISA technique.

Figure 1: Assay validation.
Vargatef (BIBF1120) is a potent inhibitor of the phospho-FGF-R2 signal detected in Kato-III cells. Highly reproducible IC_{50} values were generated in the cellular FGF-R2. The graph shows representative results.



➢ 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.