Cellular Phosphorylation Assay for ALK

CELLULAR ASSAY SERVICES

➢ The Target

The anaplastic lymphoma receptor tyrosine kinase ALK, which is highly expressed in the neonatal brain, belongs to the Insulin receptor superfamily. The kinase is involved in the development and maintenance of the central and peripheral nervous system. In most anaplastic large-cell non-Hodgkin's lymphomas developed from activated T lymphocytes, a (2;5) chromosomal translocation is found, which causes a fusion of the nucleophosmin gene NPM to the catalytic domain of ALK (NPM-ALK). This gene fusion results in a constitutive ligand-independent kinase activation.

➢ Cellular Phosphorylation Assay

The human T cell lymphoma cell line Karpas-299 carries a (2;5) chromosomal translocation, which results in the expression of an ALK variant fused to nucleophosmin (NPM-ALK). This fusion results in constitutive, ligand-independent receptor tyrosine autophosphorylation of ALK, which can be reduced by adding ALK specific inhibitors like Crizotinib (see Fig. 1). In the cellular phosphorylation assay levels of phospho-ALK are quantified by Sandwich-ELISA technique.

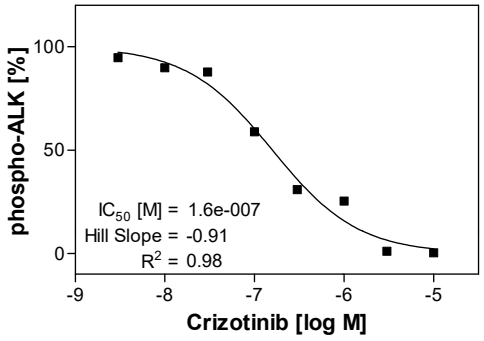
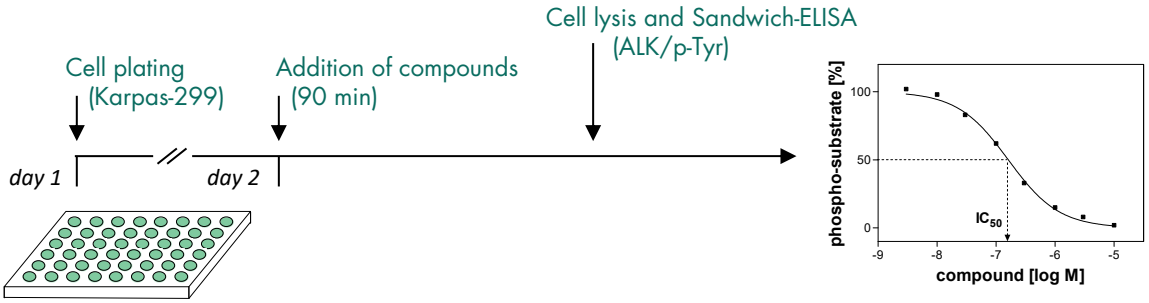


Figure 1: Assay validation.
Crizotinib potently inhibits the autophosphorylation of ALK in the described cells with highly reproducible IC_{50} values. The graph shows the result of a representative experiment.

➢ 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.