Cellular Phosphorylation Assay for IGF1-R

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

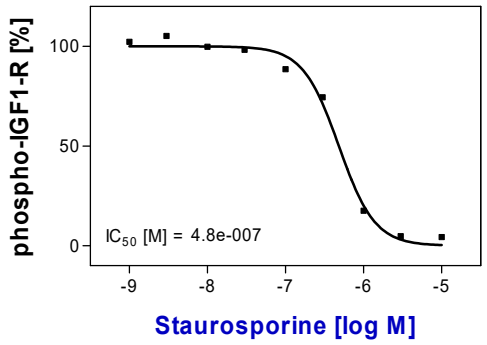
➢ The Target

The mitogenic signaling in mammalian cells is carried out mainly by growth factors that interact with receptors localized at the plasma membrane. Most of these receptors have a tyrosine kinase activity domain that is localized at the cytoplasmic region of the molecule. The interaction of the growth factors with the receptors, besides inducing the kinase activity of the receptor, activates signaling pathways that alter gene expression patterns and induce mitogenesis, or if deregulated are related to cancer. Among these receptors IGF1-R has been characterized as target for directed therapy.

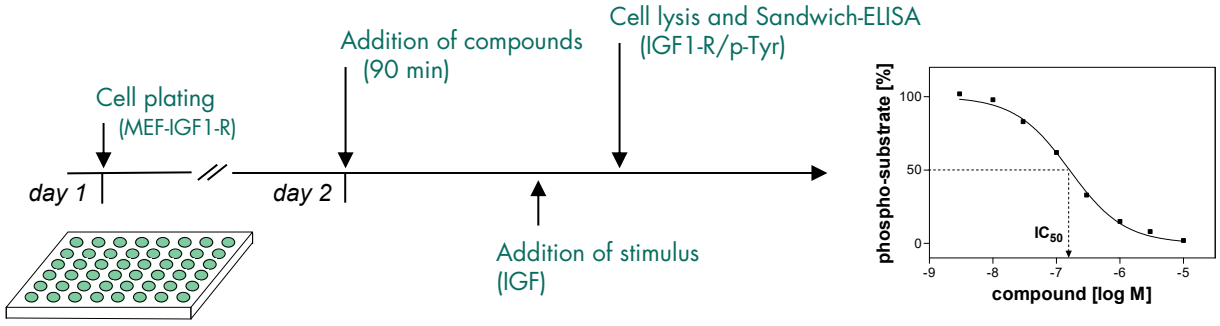
➢ Cellular Phosphorylation Assay

In the cellular IGF1-R phosphorylation assay the murine embryonal fibroblast cell line (MEF) is used, which expresses a high level of exogenously introduced full-length human IGF1-R. Stimulation of these cells with the physiological ligand human Insulin like growth factor 1 (IGF1), results in a robust IGF1-R autophosphorylation. Compounds are preincubated before cell stimulation to allow thorough target binding. Stimulation conditions are optimized to determine dose-related inhibition of the phospho-IGF1-R signal, which is subsequently quantified by Sandwich-ELISA technique. The assay is validated based on known inhibitors of IGF1-R kinase activity (see. Fig.1).

Figure 1: Assay validation.
Staurosporine blocks IGF1-R as a broad kinase inhibitor and inhibits the cellular phospho-IGF1-R response with highly reproducible IC_{50} values. The graph shows a representative result.



➢ 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.