Cellular Phosphorylation Assay for KIT

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

➢ The Target

KIT, also called SCF-R or CD117, is a cytokine receptor expressed on the surface of hematopoietic stem cells as well as other cell types. This receptor binds the stem cell factor (SCF). Mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease and acute myelogenous leukemia. Blockade of KIT by imatinib mesylate has been successfully used in the treatment of chronic myelogenous leukemia and gastrointestinal tumors. KIT overexpression in tumors indicates a possible role of KIT in tumor progression and a potential use of KIT as a target in anti-cancer therapy.

➢ Cellular Phosphorylation Assay

M07e is a human acute megakaryoblastic leukemia cell line known to overexpress the KIT receptor tyrosine kinase. Stimulation of M07e with SCF, the physiological ligand of KIT, results in a robust receptor autophosphorylation. Compounds are preincubated before cell stimulation to allow thorough target binding. Stimulation conditions are optimized to determine dose-related inhibition of the phospho-KIT signal, which is subsequently quantified by Sandwich-ELISA technique. The assay is validated based on known inhibitors of KIT kinase activity (see. Fig.1).

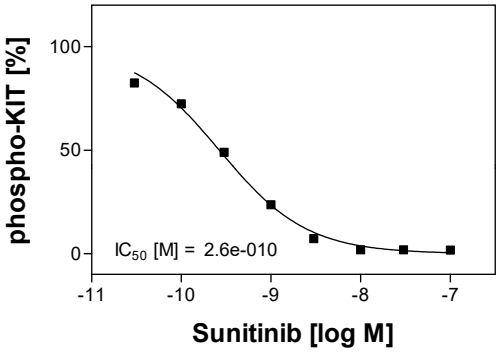
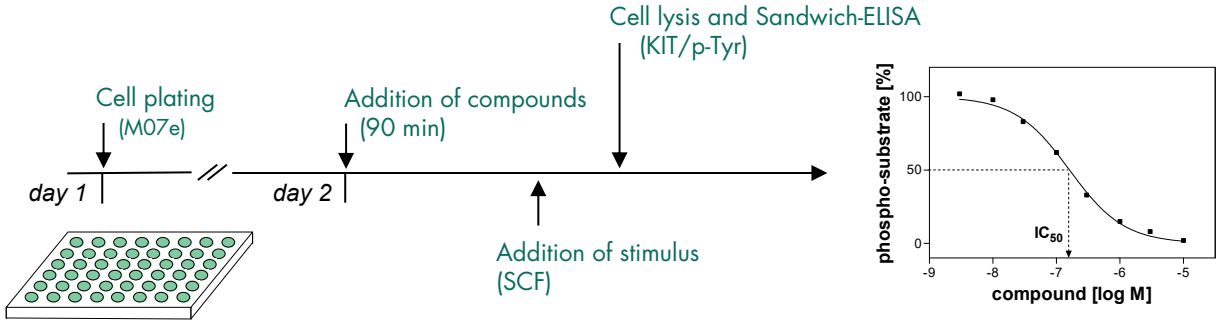


Figure 1: Assay validation.
Sunitinib is known to inhibit the SCF-induced phospho-KIT signal. The compound was included in the validation process and the cellular KIT assay generated highly reproducible IC_{50} values. The graph shows a representative result.

➢ 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.