# Cellular Phosphorylation Assay for RON

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

## ➢ The Target

The receptor tyrosine kinase RON is a also known as MST1R (macrophage stimulating 1 receptor [also known as "c-met-related tyrosine kinase"]). The primary single chain precursor protein is post-translationally cleaved to produce alpha and beta subunits, which are disulfide linked to form the mature cell-surface receptor. Binding of the ligand MSP (macrophage-stimulating protein) activates receptor autophosphorylation. RON activity regulates cell survival, migration and differentiation of epithelial cells during wound healing and promotes migration and phagocytic activity of macrophages in the innate immune response.

## ➢ Cellular Phosphorylation Assay

The human breast cancer cell line T47D is known to express high levels of RON. Stimulation of these cells with its physiological ligand macrophage-stimulating protein (MSP) 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-RON signal, which is subsequently quantified by Sandwich-ELISA technique. Figure 1 shows data confirming the inhibitory activity of Crizotinib, a cognate RON inhibitor.

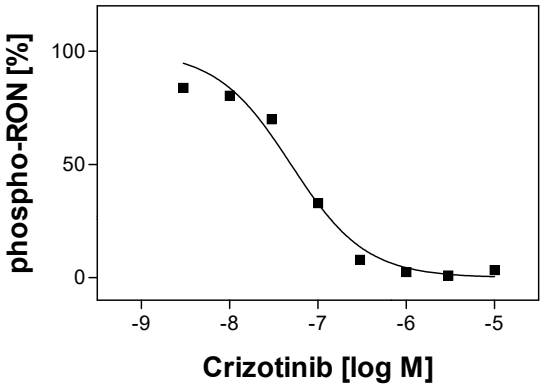
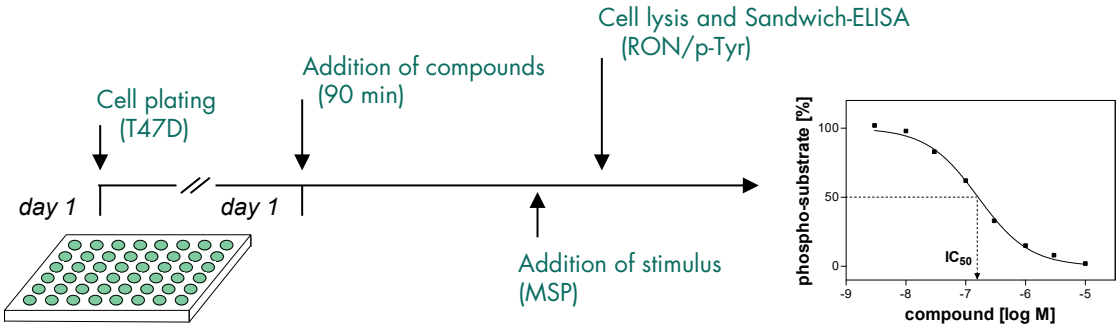



***Figure 1: Assay validation.***
*Crizotinib is a potent inhibitor of the phospho-RON signal as observed in T47D breast cancer cells. The graph shows a representative result.*

## ➢ 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.