Cellular Phosphorylation Assay for B-RAF-VE

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

➢ The Target

B-RAF is a cytosolic serine-/threonine kinase which is mutated at a high frequency in human cancer. The constitutively active mutant B-RAF V600E is found in approximately 70% of human melanomas and many other cancers. B-RAF is a component of the conserved RAS-RAF-MEK-ERK-MAP kinase pathway that regulates cellular responses to extracellular growth signals. B-RAF interacts with the small G-protein Ras resulting in translocation to the plasma membrane and activating phosphorylation at Thr^{598} and Ser^{601}. Subsequently, B-RAF phosphorylates MAPK-kinase MEK1 at Ser^{218} and Ser^{222}, thereby activating MAP-kinases (ERKs) and downstream transcription factors that drive proliferation and survival. Inhibitors of B-RAF kinase activity such as Sorafenib (Nexavar, Bay43-9006) are developed for therapeutic application.

➢ Cellular Phosphorylation Assay

Rat1 cells were transduced with B-RAF mutant B-RAF V600E which exhibits constitutive kinase activity. B-RAF V600E was fused to the N-terminus of a modified estrogen receptor (ER) which allows B-RAF activity only in the presence of estrogen-analogue Hydroxy-Tamoxifen (OHT)[1]. OHT-induced activity results in MEK1 phosphorylation and subsequent downstream effects such as ERK-phoshorylation, proliferation and soft agar growth. MEK1 phosphorylation in lysates is detected by phospho-MEK1-$Ser^{218/222}$-specific Sandwich-ELISA. The assay is validated using known inhibitors of B-RAF kinase activity (see Fig.1).

[1] Pritchard, CA et al. (1995) Mol Cell Biol **15,** 6430.

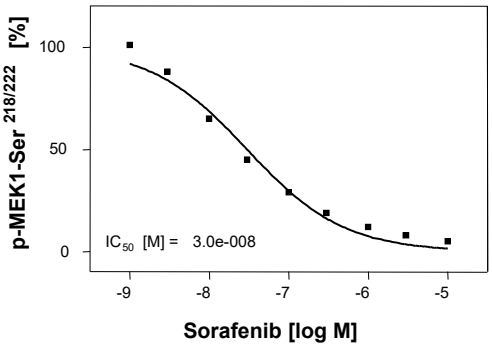
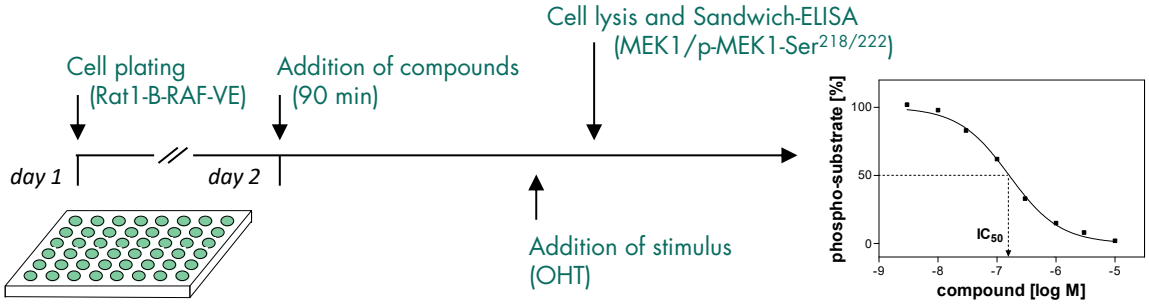


Figure 1: Assay validation.
The known B-RAF inhibitor Sorafenib blocks B-RAF V600E and inhibits the cellular phospho-MEK1-$Ser^{218/222}$ response with highly reproducible IC_{50} values. The graph shows the result of a representative experiment.

➢ 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.