Cellular Phosphorylation Assay for PIM3

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

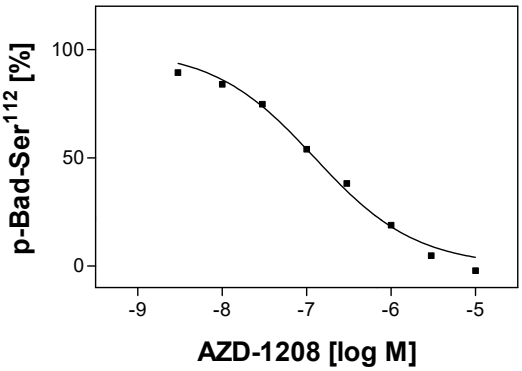
➢ The Target

Pim kinases are constitutively active serine/threonine kinases that promote growth factor-independent proliferation by phosphorylation and subsequent inhibition of a range of cellular proteins. Activity of Pim kinases is physiologically regulated via protein stability. Especially Pim1 and Pim2 kinase have been found to be overexpressed or mutated in a variety of human tumors including B cell lymphomas, leukemias, prostate cancer and oral cancer.

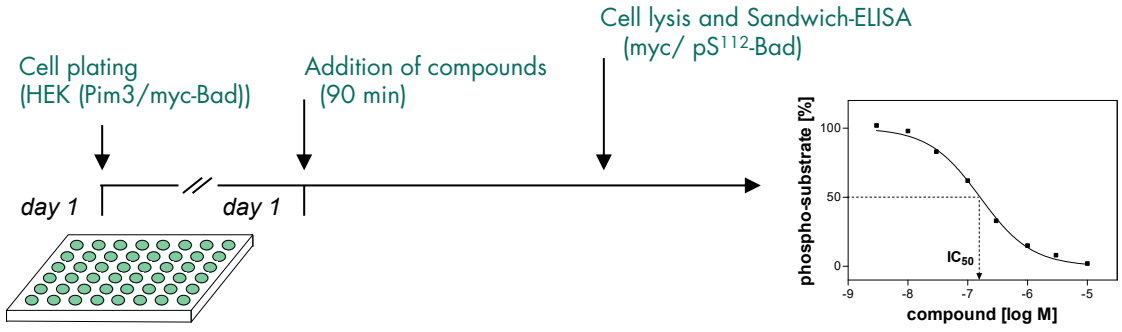
➢ Cellular Phosphorylation Assay

Transiently transfected Human embryonic kidney cells (HEK) express full length Pim3 kinase as well as Pim-substrate Bad labeled with a myc-tag. Constitutive activity of exogenous Pim3 results in phosphorylation of exogenous Bad at Ser^{112}. Upon compound incubation, cells are lysed and Bad phosphorylation is quantified by Sandwich-ELISA technique. The assay is validated based on the cognate inhibitor of Pim kinases AZD1208 (see Fig.1).

Figure 1: Assay validation.
Cognate Pim inhibitor AZD1208 blocks Pim3 and inhibits phosphorylation of Bad at Ser^{112} 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.