Cellular Phosphorylation Assay for VEGF-R2

REACTION BIOLOGY

CELLULAR ASSAY SERVICES

➢ The Target

VEGF-R2 is almost exclusively expressed on endothelial cells. It transduces the action of the well characterized pro-angiogenic vascular endothelial growth factor (VEGF-A). In pathological situations that involve neovascularization as well as enhanced vascular permeability the VEGF/VEGF-R2 signaling is responsible for activated endothelium at the tumor site. Today, VEGF-R2 is a validated target for cancer therapeutics addressing solid tumors with induced blood vessel formation.

➢ Cellular Phosphorylation Assay

Immortalized human umbilical vein endothelial cells (HUE) are known to overexpress human VEGF-R2. Stimulation of these cells with its physiological ligand VEGF-A, 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-VEGF-R2 signal, which is subsequently quantified by Sandwich-ELISA technique. The assay is validated based on known inhibitors of VEGF-R2 kinase activity (see Fig. 1).

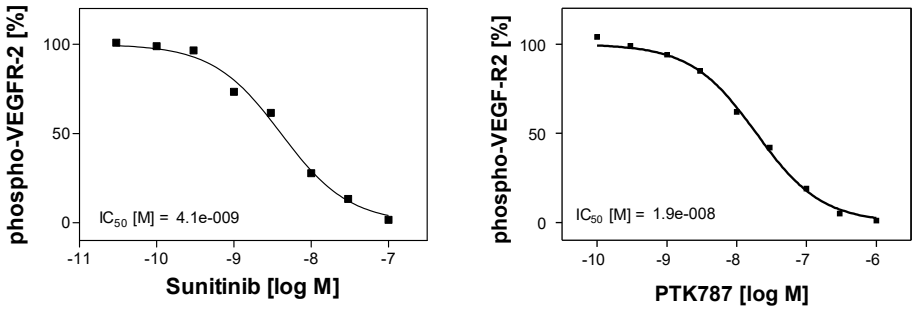
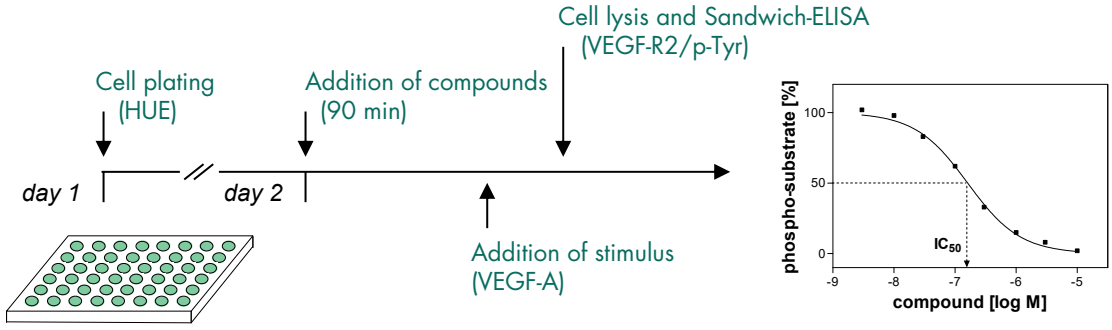


Figure 1: Assay validation.
Whereas Sunitinib blocks VEGF-R2 as a broad kinase inhibitor, PTK-787[1] is known to inhibit the VEGF-induced phospho-VEGF-R2 signal in a highly specific manner. Both compounds were included in the validation process and the cellular VEGF-R2 assay generated highly reproducible IC_{50} values. The graphs show representative results.
[1] Wood, JM et al. (2000) Cancer Res. 60(8):2178-89.

➢ 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.