

➤ Angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing ones, is a physiological process during growth and development. Beyond that angiogenesis is mandatory for tumor growth and is involved in other pathological disorders (e.g. psoriasis, macular degeneration). The angiogenic cascade can be divided in at least five stages: matrix degradation, endothelial cell (EC) migration, EC proliferation, lumen formation and vessel maturation. The cellular angiogenesis assay mimics the in vivo situation and the first stages of the angiogenic cascade are involved. Despite its complexity the cellular angiogenesis assay demonstrates a high degree of reproducibility and robustness.

➤ Assay Procedure

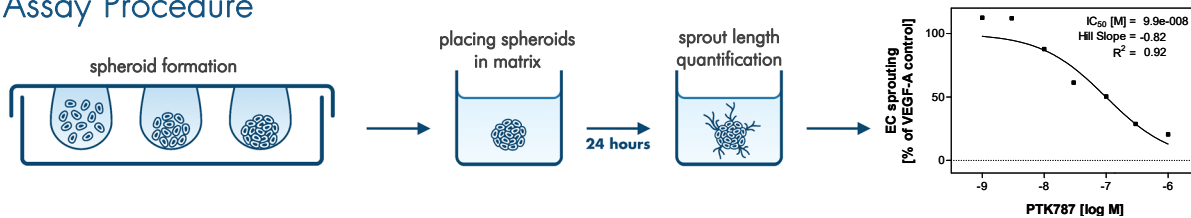


Figure 1: Assay procedure. Human EC spheroids (fibroblasts and smooth muscle cell spheroids may also be used) are mixed in a collagen or fibrin matrix and transferred into 24 well plates. After polymerization the test compounds and/or stimulation factors (VEGF-A, FGF-2, Deferoxamine, HB-EGF, etc.) are added. The assay is incubated at 37 °C for 24 h and subsequently fixed. EC sprouting is quantified by measuring the sprout length and the cumulative sprout length (CSL) is determined.

➤ Assay Features

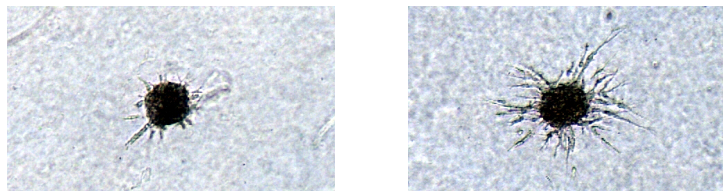


Figure 2: Assay features. Unstimulated EC spheroids show limited sprouting into the surrounding collagen matrix (left). EC sprouting can be induced by pro-angiogenic factors like VEGF-A (right). The number and length of the sprouts corresponds to the angiogenic activity. Pro-angiogenic compounds induce 3D EC sprouting originating from the spheroid whereas angiogenic inhibitors prevent sprout formation induced by pro-angiogenic factors (e.g. VEGF-A, FGF-2).

➤ Study Example

Figure 3: Study example. The angiogenesis inhibitors PTK787 and Sunitinib were tested for inhibition of EC sprouting (VEGF-A and Deferoxamine induced) and fibroblast scattering. The selective inhibitor PTK787 is active on EC sprouting but does not affect fibroblast scattering. In contrast Sunitinib which has a broader kinase inhibition profile than PTK787 inhibits both EC sprouting and fibroblast scattering.

