

Renal mesangial cell fibrosis assay

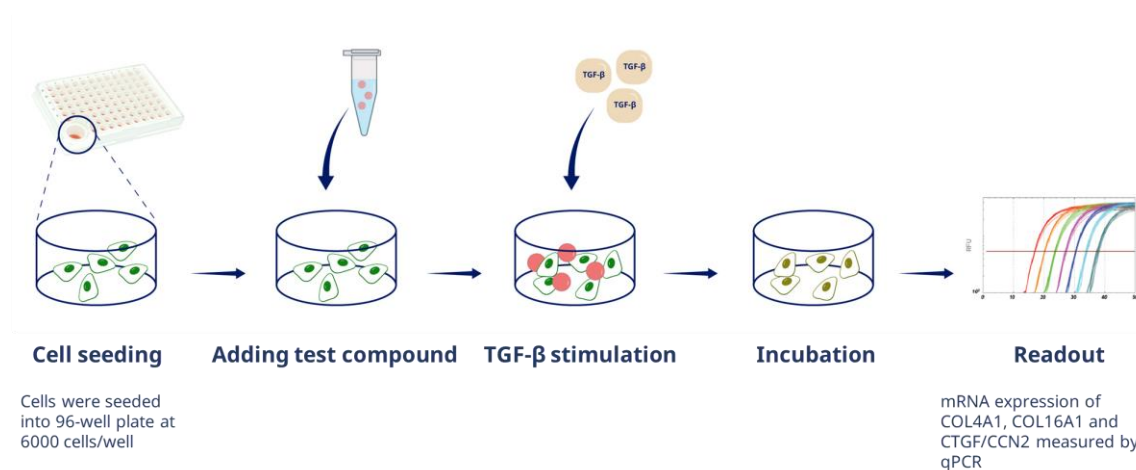
Disease relevance

Mesangial cells comprise approximately one-third of kidney glomerular cell population. Fibrotic ECM generated by the mesangial cells play an important role in the progression of chronic kidney disease (CKD).

Assay set-up

Primary human mesangial cells are cultured in 96-well plate in basal condition or stimulated by TGF- β to induce fibrosis.

We will test your compounds for its ability to promote or inhibit fibrosis in basal and in fibrotic condition. And the readout will be mRNA regulation of fibrotic genes (COL4A1, COL16A1 and CTGF/CCN2).



Experimental protocol

Day 1, HRMCs are seeded into a 96-well plate at 6000 cells/well in culture medium containing FBS.

Day 2, the tested compounds and the corresponding control (only when the test compounds are siRNA / ASO compounds) will be transfected into cells by Lipofectamine® RNAiMAX according to the manual in two different concentration (2, 10 nM for siRNA, 100, 250 nM for ASO)

Day 3, cell culture medium is replaced with starvation medium (0.5% FBS) for a 24-hour incubation.

Day 4, cells are treated with or without TGF- β in fresh starvation medium for another 24 hours. And cells may be co-treated with the test compounds (only when the test compounds are proteins / peptides / small molecules) in titrated concentration. Vehicle of the test compounds will be used as negative control. Cells will be incubated for another 24 hours

Day 5, cells are lysed for total RNA extraction and the mRNA levels of the fibrotic genes (COL4A1, COL16A1 and CTGF/CCN2) are measured by qPCR.