

Hepatic Stellate Cell Fibrosis Assay

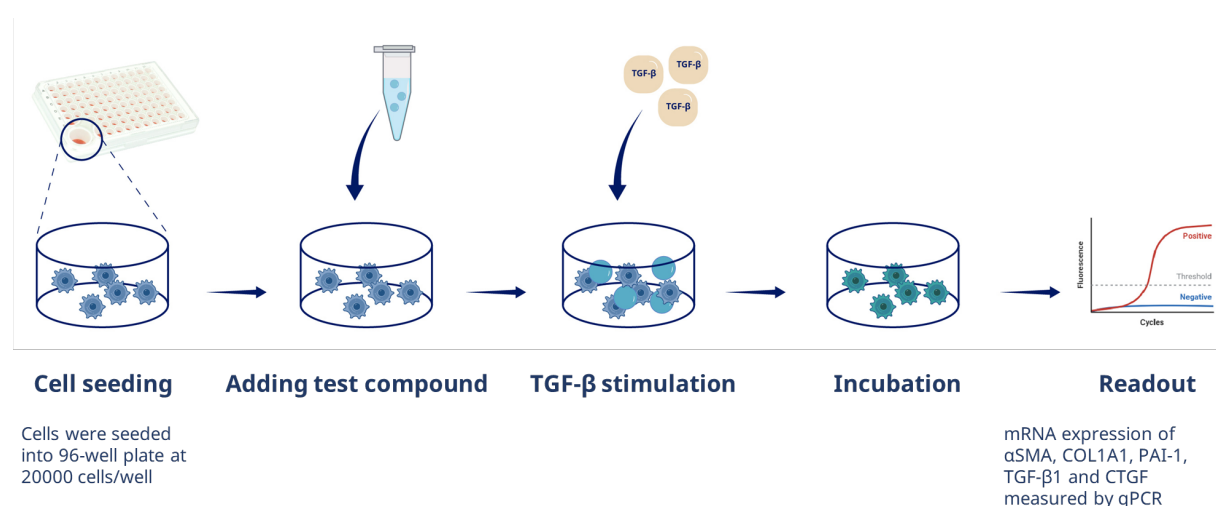
Disease relevance

Hepatic stellate cells (HSCs) are a major fibrogenic cell type that contributes to collagen accumulation during chronic liver disease. The LX-2 human hepatic stellate cell line has been extensively characterized and retain key features of hepatic stellate cytokine signalling, neuronal gene expression, retinoid metabolism, and fibrogenesis, making them a highly suitable model of human hepatic fibrosis.

Assay set-up

Hepatic stellate cells are cultured in 96-well plate in basal state 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 (α SMA, COL1A1, PAI-1, TGF- β 1 and CTGF).



Experimental protocol

On day 0, LX-2 cells will be seeded into 96 well plate or 384 well plate in culture medium with 2% FBS.

For peptide/protein/small chemicals, cells will be treated with compound at indicated concentrations on day 1 for 30 min, then add TGF- β to a final concentration of 5ng/mL in the assay medium, 200 μ L/well (50 μ L/well for 384 well plate). Incubate at 37°C incubator with 5% CO₂.

For siRNA or ASO compounds, cells will be transfected with siRNA/ASO on day 1. TGF- β will be added on day 3 to a final concentration of 5ng/ml in the assay medium, 200 μ L/well (50 μ L/well for 384 well plate). Incubate at 37°C incubator with 5% CO₂.

After 24 h stimulation by TGF- β , cells will be lysed, total RNA will be extracted and mRNA level of fibrotic markers (α SMA, COL1A1, PAI-1, TGF- β 1 and CTGF) will be measured by qPCR.