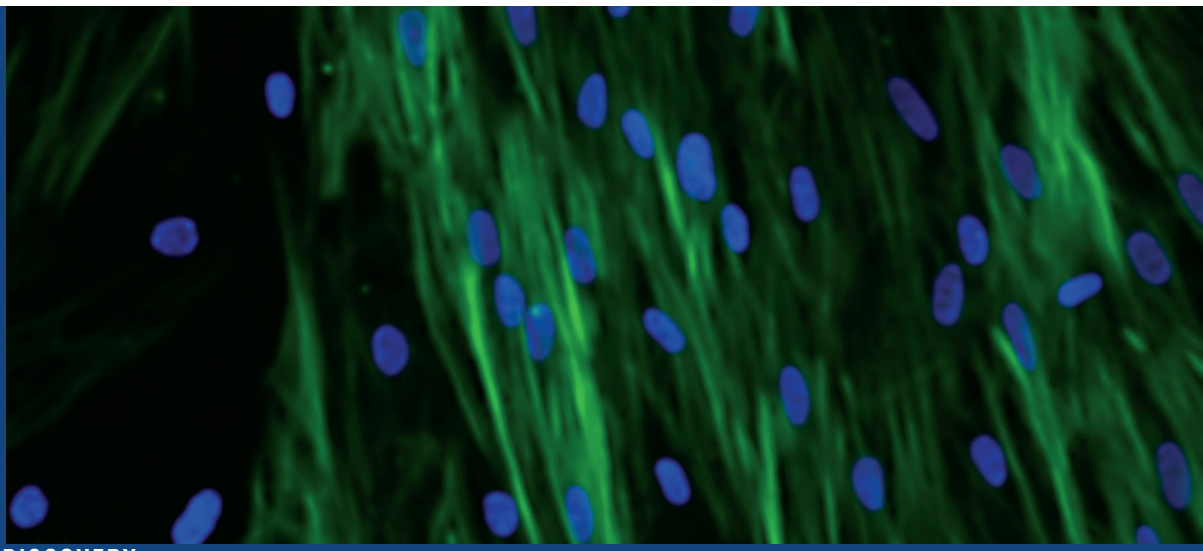
charles river

Overview

Fibrosis results from misregulated complex pathways involving multiple cell types such as epithelial cells and fibroblasts. We've developed an optimized, off-the-shelf panel of *in vitro* fibrosis assays using our own patient-derived donor cells to assess the translational potential of small molecules as novel therapies.



DISCOVERY

Off-the-Shelf Assay:

Complex Biology *In Vitro* Assays: Fibrosis

Fibroblast-to-Myofibroblast Transition (FMT) Assay

Click to learn more

Fibroblast-to-Myofibroblast Transition (FMT) Assay in Human Lung Cells Derived from Idiopathic Pulmonary Fibrosis (IPF) Patients and Healthy Donors

A well-characterized hallmark of pathologic FMT is *de novo* formation of alpha-smooth muscle actin (αSMA) stress fibers. Since myofibroblasts localize at sites undergoing active matrix deposition and display elevated collagen synthetic capacity, myofibroblasts are considered to play a major role in the pathology of idiopathic pulmonary fibrosis (IPF). The well-established key fibrogenic mediator, transforming growth factor TGF-β1, induces FMT. In cells that have undergone FMT, increased expression of αSMA is observed. *In vitro*, increased αSMA expression positively correlates with contraction of myofibroblast populated collagen gels, indicating that αSMA is a strong marker of myofibroblast differentiation and hence, a relevant readout for lung fibrosis. A validated, robust TGF-β1-induced FMT cell-based assay has been developed in IPF-derived fibroblasts to evaluate therapeutic candidates with various modes-of-action in this disease area.

Need a custom version of this assay?

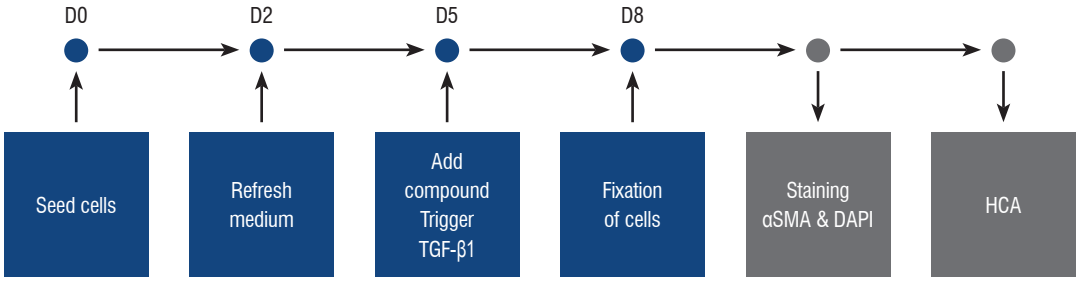
Visit criver.com/ds-vitro-assay

Need a custom version of this assay?

Visit criver.com/ds-vitro-assay

FMT Assay Principle

Lung-derived primary human bronchial fibroblasts are seeded then refreshed in preparation for addition of small molecule compounds and the TGF-β1 trigger. After 3 days, the cells are fixed, then stained using DAPI-labeled αSMA and imaged via high-content analysis (HCA).



FMT Assay Setup

FMT protocol has been developed for analysis of trans-differentiation of fibroblasts to myofibroblasts. Marker expression is quantified using in-house developed algorithms on a HCA platfrom.

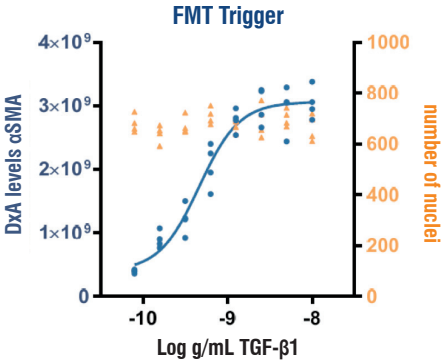
- Cells → lung fibroblasts from IPF donors or healthy donors
- Seeding density → 3,000 cells/well in 96-well plates
- Trigger → 1.25 ng/mL TGF-β1
- Assay controls → 0.1% DMSO (negative control) and 1 µM SB525334 (positive control)
- Compounds → 8-point concentration response curves (in biological duplicate)
- Fix → 72 hours post-trigger
- Readout → αSMA and DAPI staining (high-content analysis)

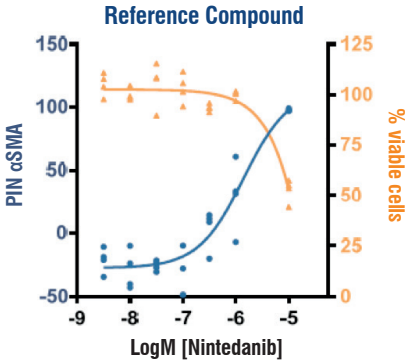
Assay Performance

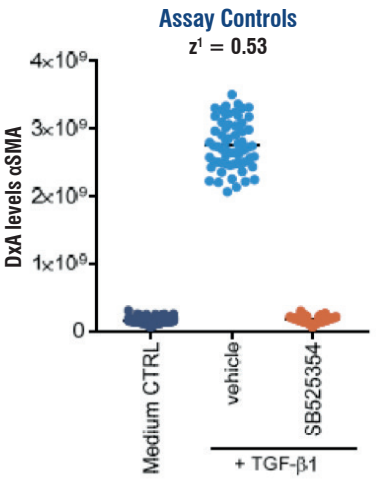
Need a custom version of this assay?

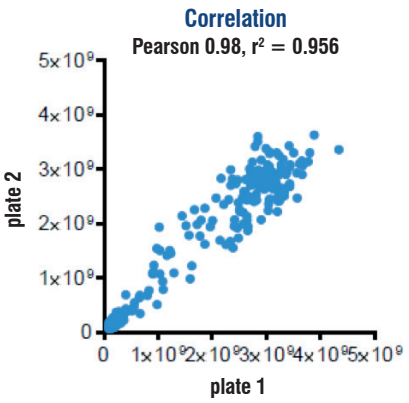
Visit criver.com/ds-vitro-assay

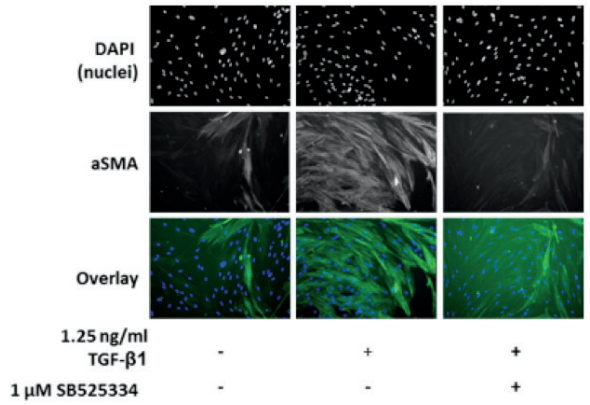
Representative concentration response data shown below from patient-derived fibroblasts, 72 hours post TGF-β1 trigger.











Need a custom version of this assay?

Visit criver.com/ds-vitro-assay

Summary

Lung-derived fibroblasts stimulated with TGF-β1 demonstrated a clear concentration-dependent increase in αSMA levels, while increasing the TGF-β1 stimuli showed no effect of on the number of nuclei, indicative of no cytotoxic events. TGF-β1 trigger could be inhibited by treatment with an ALK-5 inhibitor, showing full inhibition of αSMA regardless of the presence of TGF-β1. IC50 values were consistent between different donors, and strong Pearson correlation denotes consistency between biological replicates. Using these fibrosis assays, trans-differentiation FMT can be monitored to evaluate therapeutic candidates.

The therapeutic candidates can be evaluated in 8-step CRC using three different IPF patients/healthy donors in biological duplicate for their effect on αSMA modulation. In addition, potential cytotoxic side-effects of the tested therapeutic candidate will be assessed by monitoring the loss of nuclei as a measure for cell death. Results will be provided as percentage inhibition (PIN values) and % viable cells.

For our clients' scheduling convenience, we perform FMT assays on a routine bi-monthly basis. Results are issued within 6–8 weeks of receipt due date.

FMT Assay – Compound Receipt Due Dates

May 2020	August 2020	November 2020
4	14	13

Assay Reference codes

Fibroblast-to-Myofibroblast Transition (FMT) Assay – IPF Human-Derived Donor Cells
Assay reference code: OTS101-FMT-LUNG-IPF

Fibroblast-to-Myofibroblast Transition (FMT) Assay – Healthy Human-Derived Donor Cells
Assay reference code: OTS102-FMT-LUNG-HEALTHY

Complementary Fibrosis Assays

Epithelial-to-Mesenchymal Transition (EMT) Assay

M1 Polarization Assay

M2 Polarization Assay

© 2019, Charles River Laboratories International, Inc.