FUJIFILM
Value from Innovation

Cellular Dynamics

Detecting Fibrosis with iCell® Cardiac Fibroblasts

iCell Lab Note

Introduction.

Human induced pluripotent stem cell (iPSC)-derived cardiac fibroblasts have become an invaluable tool for studying cardiac fibrosis, a pathological condition commonly associated with various cardiovascular diseases. This iCell® Lab Note highlights the development of an image-based fibrosis assay using iCell Cardiac Fibroblasts. The data demonstrate the activation of cardiac fibroblasts using TGF-β1 to replicate the fibrotic response observed in the heart, as well as the suppression of fibrotic phenotypes through treatment with an ALK5 inhibitor. These results were generated in collaboration with PhenoVista Biosciences, utilizing iCell Cardiac Fibroblasts cultured in iCell Cardiomyocytes Maintenance Medium supplemented with the iCell Cardiac Co-Culture Supplements Kit.

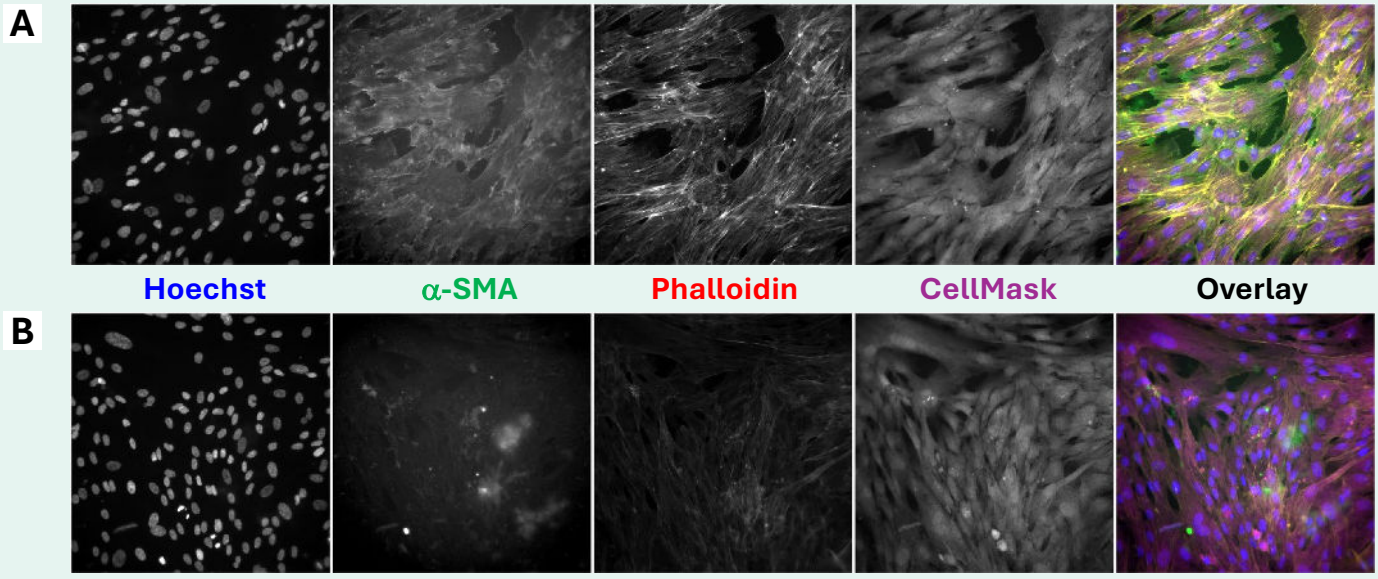


Figure 1. High Content Imaging. iCell Cardiac Fibroblasts, 11713 were plated in a 384-well assay plate. The next day, cells were pre-treated ± ALK5 inhibitor (10 µM) for 60 min and then stimulated with TGF-β1 (30 ng/ml) for 72 hours. Plates were fixed, stained, and scanned on a CellInsight CX7 LED Pro HCS platform (Thermo Fisher) with a 20x objective. Fluorescent stains used were Hoechst (405 nm; blue), α-SMA (488 nm; green), Phalloidin (568 nm; red), and CellMask Deep Red (647 nm; violet). *Images provided by PhenoVista Biosciences.*

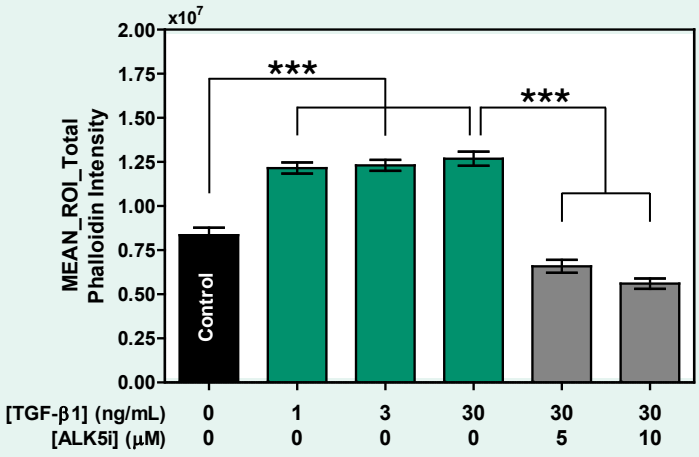
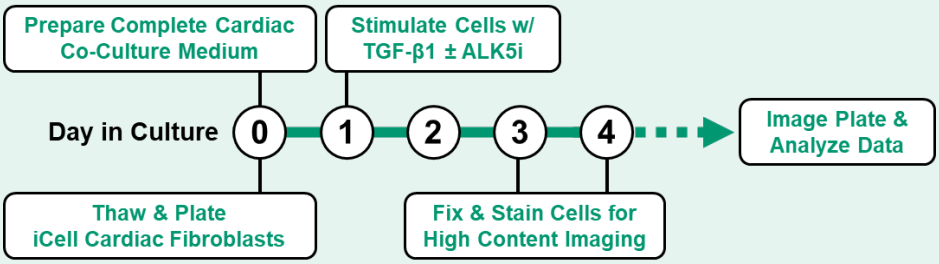
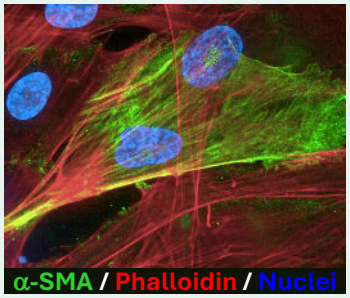


Figure 2. Image-based Analysis of Fibrosis. Using the assay workflow described in this iCell Lab Note, image-based analysis was conducted on 3–6 wells per condition, with 9 fields assessed per well. Total phalloidin intensity revealed significant differences between untreated iCell Cardiac Fibroblasts (control, shown in black), cells stimulated with TGF-β1 (1, 3, or 30 ng/mL, shown in green), and those treated with ALK5 inhibitor (TGFβR1 inhibitor; 5 or 10 µM, shown in gray). Additional image-based metrics such as fiber alignment, cell area for α-SMA or phalloidin, and nuclear count were also evaluated (data not shown). Significant differences ($p < 0.001$, determined by Student's t-test) between control and treated conditions are denoted with (***). All analyses were performed by PhenoVista Biosciences.

Assay Workflow Schematic.





Methods.

Refer to iCell Cardiac Fibroblasts Quick Guide for info on storing and handling cells, media, and supplements.

- Combine iCell Cardiomyocytes Maintenance Medium (100 ml) with iCell Cardiac Co-Culture Supplements A (25 ml) and B (250 µl) to make Complete Cardiac Co-Culture medium.
- Coat 384-well cell culture plate with vitronectin (VTN-N) according to manufacturer's recommendations.
- Thaw cells and resuspend cell suspension in Complete Cardiac Co-Culture Medium to 40,000 cells/ml using the number of viable cells/vial listed on the CoA.
  - Visit www.fujifilmcdi.com/coa-lookup/
- Dispense 32 µl of cell suspension into each well of a 384-well plate to achieve a density of ~1280 cells/well.
- Incubate plate overnight at 37ºC and 5% CO_2.
- Treat cells with ALK5 inhibitor (Galunisertib) for 60 min prior to stimulation with TGF-β1 (for 48-72 hours).
- Fix cells and stain for fluorescent markers on imaging system, such as the CX7: Hoechst (405 nm), α-SMA (488 nm), Phalloidin (568 nm), and CellMask (647 nm).

There are multiple ways to detect induction of fibrotic markers, include qPCR, ELISA, and high content imaging. Contact FCDI for more information.

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell Cardiac Fibroblasts Kit, 11713 [1]	FCDI	R1256
▪ iCell Cardiomyocytes Maintenance Medium	(incl. in kit)	M1003
iCell Cardiac Co-Culture Supplement Kit	FCDI	R1258
▪ iCell Cardiac Co-Culture Supplement A	(incl. in kit)	M1047
▪ iCell Cardiac Co-Culture Supplement B	(incl. in kit)	M1050
Recombinant Human Vitronectin (VTN-N)	Thermo Fisher	A14700
Recombinant Human TGF-β1 [2]	R&D Systems	7754-BH
Galunisertib (ALK5/TGFβR1 inhibitor)	Tocris	6956
Anti-alpha Smooth Muscle Actin Mouse mAb [3]	Abcam	AB7817
AlexaFluor™ 568 Phalloidin	Thermo Fisher	A12380
Hoechst 33342 Solution	Thermo Fisher	H3570

[1] Human iPSC-derived cardiac fibroblasts from female donor 01434 are also available as Catalog # R1257
[2] Other sources of TGF-β1 may also work in this assay but have not been tested.
[3] Gene name for this target is ACTA2. Clone ID for this mAb is 1A4.

Summary.

This iCell Lab Note provides example data and protocol guidance for using high content imaging to measure fibrotic phenotypes induced by TGF-β1 in iCell Cardiac Fibroblasts. Importantly, iCell Cardiac Co-Culture Supplements are required to enhance the responsiveness of the cells to this treatment. As demonstrated by ALK5i modulation, this approach offers a biologically-relevant platform to investigate cardiac fibrosis mechanisms and to develop targeted interventions that mitigate fibrotic processes in the heart.

Highlights.

iCell Cardiac Fibroblasts are human cells that can be produced in large batches with high lot-to-lot consistency.

iCell Cardiac Fibroblasts are highly pure cells that are quiescent at thaw and can be activated using TGF-β1 to generate fibrotic phenotypes.

PhenoVista Biosciences helped to develop this image-based cardiac fibrosis assay in 384-well format to enable dose-response studies and be compatible with HTS screening.

Scan here to download the iCell Cardiac Fibroblasts Quick Guide.

Contact **Technical Support** for more protocol details and supportive data.
FCDI-Support@fujifilm.com

www.fujifilmcdi.com
FUJIFILM Cellular Dynamics, Inc.
(608) 310-5100 | Toll-free US (877) 310-6688

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