InvivoSciences, Inc.

Discovery with a Human Touch

Quantify Filamentous Actin (F-Actin)

Application:

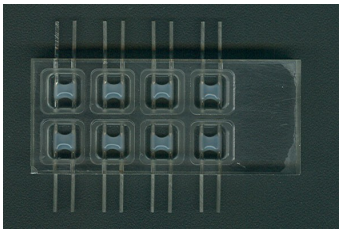
Assess and monitor the integrity of the actin cytoskeleton. Cells embedded in three-dimensional (3D) tissue constructs are fixed, permeabilized, and then labeled with fluorophore-conjugated phalloidin which targets filamentous actin (F-actin). Fluorescence intensity in each tissue construct is then quantified with a plate reader.

Introduction:

The actin cytoskeleton is responsible for bearing tension and maintaining cellular shapes. This is important for cell motility, contraction, and cytokinesis. Disruption in F-actin formation and maintenance prevents force generation, alters cell physiology, and can lead to cell death. Quantitative monitoring of cellular F-actin content is thus informative for various biomedical research fields including cell biology and cancer research.

Technical Advantages:

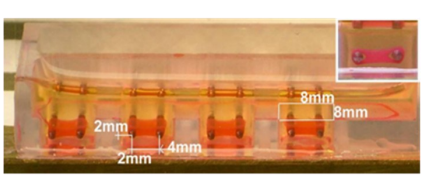
- Rapidly assess cellular F-actin content using cells grown in a three-dimensional (3D) environment which is more natural than the commonly used two-dimensional (2D) cell culture surfaces
- Correlate F-actin content with multiple parameters of cell and tissue physiology, including viability and contractility (as assessed with MTT and InvivoSciences, LLC's proprietary Palpator™ technologies)
- Culture and re-asses 3D tissue constructs for days and weeks to prolong experiments and repeatedly achieve accurate results



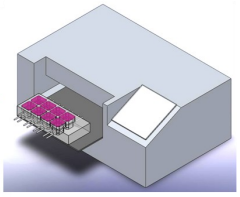
- Correlate F-actin studies with multiple parameters of cell physiology including cellular contractility and viability

Example:

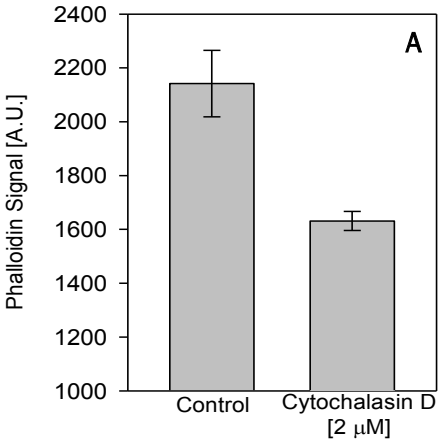
The 3D tissue constructs were treated with cytochalasin D (CD, 2 μM) for 24 hours and then fixed with 4% formaldehyde for 1 hour. The tissues were then treated with 0.1% TritonX for 30 minutes and labeled with Alexa 568™ conjugated phalloidin for 1 hour. Fluorescent signal intensity was read with a plate reader.

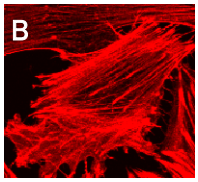


•Indicator staining
•Drug treatment

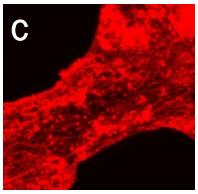


Signal reading





Control



CD [2 μM]

CD treatment significantly reduced Phalloidin signals indicating F-actin content in the 3D tissue constructs (Figure A). The differences in F-actin were confirmed visually with the loss of F-actin in CD-treated cells (Figures B and C).

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