

Protocol for Emulate Organ-Chips:

Immunofluorescence Staining of F-actin

March 7, 2019

EP151 v1.0



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| Goals: | Key Steps: | Other Required Materials: |
|---|--|---|
| Visualize F-actin via fluorescent imaging in Emulate Organ- Chips | Immunofluorescence staining in chip | Alexa Fluor™ 488 Phalloidin (Invitrogen™ A12379) Dimethyl sulfoxide (DMSO) PBS 4% paraformaldehyde (PFA) 10% Saponin PBS BSA Fluorescence microscope |

1. Method

| Sample type | Fixed Organ-Chip See Protocol EP137 Fixation and Immunofluorescence (IF) Staining. | | |
|---|---|--|--|
| Recommended fixative and incubation time | 4% PFA, 15 minutes at room temperature | | |
| Recommended permeabilization and incubation time | 1% saponin in PBS, 30 minutes at room temperature | | |
| Recommended blocking buffer and incubation time | 1% BSA,10% goat serum in PBS, overnight at 4°C | | |
| Recommended primary antibody dilution and incubation time | Alexa Fluor™ 488 Phalloidin (Invitrogen™ A12379) Dilute 1:1000 in PBS (stock prepared in DMSO), 30 minutes incubation at room temperature | | |
| Representative image | Image of F-actin (green) in liver sinusoidal endothelial cells in the Liver-Chip (bottom channel). | | |
| More information on vendor site | https://www.thermofisher.com/order/catalog/product/A12379 | | |





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