



emulate

Protocol for Emulate Organ-Chips:
Immunofluorescence Staining of CD31

April 8, 2019

EP163 v1.0

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Goals:	Key Steps:	Other Required Materials:
Visualize CD31 or platelet endothelial cell adhesion molecule (PECAM-1) in Organ-Chips	<ul style="list-style-type: none"> Immunofluorescence staining in Organ-Chips 	<ul style="list-style-type: none"> Anti-CD31 antibody (Abcam ab9498) 4% paraformaldehyde (PFA) 10% saponin PBS BSA Normal goat serum (or other serum from the species the secondary antibody was raised in) Alexa Fluor® 555-conjugated goat anti-mouse IgG secondary antibody (or another anti-mouse secondary antibody) Fluorescence microscope

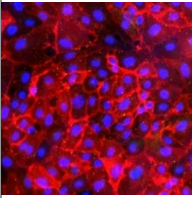
1. Background

CD31 or PECAM-1, is a type I integral membrane glycoprotein that is expressed at high levels on endothelial cells. CD31 staining can be used as a marker of endothelial cells. The expression on endothelial cells is concentrated at junctions between adjacent cells.

2. Method

Sample type	Fixed Organ-Chip See Protocol EP173 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	Anti-CD31 (PECAM-1) (Abcam ab9498) 1:50 dilution in blocking buffer, overnight at 4°C
Recommended antibody host	Mouse

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Recommended secondary antibody dilution and incubation time	Alexa Fluor® 555-conjugated goat anti-mouse IgG secondary antibody 1:500 dilution in blocking buffer, 2 hours at room temperature in the dark.
Representative image	 <p>Image of CD31 (red) / nuclei staining (blue) indicating presence of LSECs in the human Liver-Chip (bottom channel)</p>
More information on vendor site	CD31: https://www.abcam.com/cd31-antibody-jc70a-ab9498.html

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