



emulate

Protocol for Emulate Organ-Chips:  
Immunofluorescence Staining of CD68

April 9, 2019

EP156 v1.0

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Goals:	Key Steps:	Other Required Materials:
Visualize CD68, a Kupffer cell marker, in fixed Emulate Organ-Chip	<ul style="list-style-type: none"> <li>Immunofluorescence staining in chip</li> </ul>	<ul style="list-style-type: none"> <li>Anti-CD68 monoclonal antibody (KP1) (Invitrogen™, MA5-13324)</li> <li>4% paraformaldehyde (PFA)</li> <li>10% saponin</li> <li>PBS</li> <li>BSA</li> <li>Normal goat serum (or other serum from the species the secondary antibody was raised in)</li> <li>Alexa Fluor™ 555-conjugated goat anti-mouse IgG secondary antibody (or other anti-mouse secondary antibody)</li> <li>Fluorescence microscope</li> </ul>

## 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	Anti-CD68 monoclonal antibody (KP1) (Invitrogen™ MA5-13324) 1:100 dilution in blocking buffer, overnight at 4°C
Recommended antibody host	Mouse
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

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Representative image		Image of CD68 (red) / nuclei staining (blue) indicating presence of Kupffer cells in the human Liver-Chip (bottom channel)
More information on vendor site	<a href="https://www.thermofisher.com/antibody/product/CD68-Antibody-clone-KP1-Monoclonal/MA5-13324">https://www.thermofisher.com/antibody/product/CD68-Antibody-clone-KP1-Monoclonal/MA5-13324</a>	

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