

## Protocol for Emulate Organ-Chips

Immunofluorescence Staining of Stabilin-1

April 23, 2019

EP157 v1.0



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Goals:	Key Steps:	Other Required Materials:
Visualize the multifunctional scavenger receptor stabilin-1 in Emulate Organ- Chips	<ul> <li>Immunofluorescence staining in Organ- Chips</li> </ul>	<ul> <li>Anti-Stabilin-1 (STAB1) antibody (Novus NBP1- 84444)</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<sup>®</sup> 555-conjugated goat anti-rabbit IgG secondary antibody (or other anti-rabbit secondary antibody)</li> <li>Fluorescence microscope</li> </ul>

## Introduction

This gene encodes a large, transmembrane receptor protein and is primarily expressed on sinusoidal endothelial cells of liver, spleen, and lymph node. The receptor has been shown to endocytose ligands such as low density lipoprotein, Gram-positive and Gram-negative bacteria, and advanced glycosylation end products.

## Method

	Fixed Organ-Chip
Sample type	See Emulate Protocol EP137 Fixation and Immunofluorescence (IF) Staining of Emulate Organ-Chips.
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-Stabilin-1 (STAB1) (Novus NBP1-84444) 1:50 dilution in blocking buffer, overnight at 4°C
Recommended antibody host	Rabbit



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Recommended secondary antibody dilution and incubation time	Alexa Fluor <sup>®</sup> 555-conjugated goat anti-rabbit IgG secondary antibody 1:500 dilution in blocking buffer, 2 hours at room temperature in the dark	
Representative image	Image of STAB-1 (red) / nuclei staining (blue) indicating presence of LSECs in the human Liver-Chip (bottom channel)	
More information on vendor site	Stabilin-1: https://www.novusbio.com/products/stabilin-1- antibody_nbp1-84444	

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