

## Protocol for Emulate Organ-Chips

Immunofluorescence Staining of Stabilin-2

March 11, 2025

EP237 Rev A

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Goals:	Key Steps:	Other Required Materials:
Visualize the multifunctional scavenger receptor stabilin-2 in Emulate Organ- Chips	<ul> <li>Immunofluorescence staining in Organ- Chips</li> </ul>	<ul> <li>Anti-Stabilin-2 (STAB2) antibody (Abcam, ab121893)</li> <li>4% paraformaldehyde (PFA)</li> <li>0.125% Triton X-100</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 hyaluronan, low density lipoprotein, Gram-positive and Gram-negative bacteria, and advanced glycosylation end products.

## Method

	Fixed Organ-Chip		
Sample type	See Emulate Protocol <u>EP137</u> 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	0.125% Triton X-100 in PBS, 10 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-2 (STAB2) (Abcam, ab121893) 1:50 dilution in blocking buffer, overnight at 4°C		
Recommended antibody host	Rabbit		
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		

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Representative image		Image of STAB-2 (yellow) / nuclei staining (blue) indicating presence of LSECs in the human Liver-Chip (bottom channel)
More information on vendor site	Stabilin-2: <u>https://www.abcam.com/en-us/products/primary-antibodies/stabilin-2-antibody-ab121893</u>	

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