emulate

Protocol for Emulate Organ-Chips:

Immunofluorescence Staining of Alpha Smooth Muscle Actin

April 4, 2019

EP136 v1.0

Goals:	Key Steps:	Other Required Materials:
Visualize smooth-muscle actin filaments in fixed Emulate Organ-Chip	• Immunofluorescence staining in chip	• Anti-alpha smooth muscle actin (α-SMA) antibody (Abcam, ab5694) • 4% paraformaldehyde (PFA) • 10% Saponin • PBS • BSA • Normal goat serum (or other serum from the species the secondary antibody was raised in) • Alexa Fluor™ 488-conjugated goat anti-rabbit IgG secondary antibody (or other anti-rabbit secondary antibody) • 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	Anti-α-SMA antibody (Abcam, ab5694) 1:1000 dilution in blocking buffer, overnight at 4°C
Recommended antibody host	Rabbit
Recommended secondary antibody dilution and incubation time	Alexa Fluor™ 488-conjugated goat anti-rabbit IgG secondary antibody 1:500 dilution in blocking buffer, 2 hours at room temperature in the dark.

TITLE	DOCUMENT	VERSION
Immunofluorescence Staining of Alpha Smooth Muscle Actin	EP136	1.0
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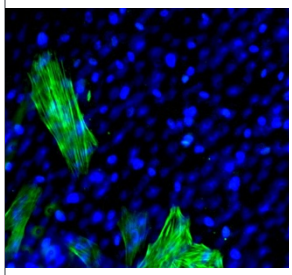
Representative image	 Image of α-SMA / nuclei staining indicating activated stellate cells in the human Liver-Chip (bottom channel)
More information on vendor site	https://www.abcam.com/alpha-smooth-muscle-actin-antibody-ab5694.html?productWallTab=ShowAll