Protocol for Emulate Organ-Chips:

Live Staining of CDFDA Uptake into Bile Canaliculi

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EP195 v1.0

TITLE	DOCUMENT	VERSION
Live Staining of CDFDA Uptake into Bile Canaliculi	EP195	1.0
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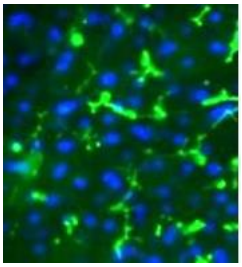
Goals:	Key Steps:	Other Required Materials:
Visualize bile canaliculi structure and MRP2 transporter function in Emulate Organ-Chips	• Live staining and fluorescent imaging in Liver-Chips	• Carboxy-DCFDA (CDFDA) (Invitrogen, Cat # C369) • PBSPBS • Fluorescence microscope

Introduction

Carboxy-DCFDA (5-(and-6)-Carboxy-2',7'-Dichlorofluorescein Diacetate) is a reagent that passively diffuses into cells. It is colorless / nonfluorescdent and is cleaved by intracellular esterases to yield a fluoresecent fluirophore, 5-(and-6)-carboxy-2',7' –dichlorofluorescein (CDFDA). This reagent can be used to visualize the formation of bile canaliculi in polarized hepatocytes which is indicative of MRP-2 trasnporter activity. Carboxy-DCFDA is a substrate of multidrug resistance-associated protein 2 (MRP2), a hepatocyte efflux transporter, thus it can be used to visualize MRP2-mediated canalicular uptake and efflux, as well as to label the bile canaliculi structures.

Method

Sample type	Live Liver-Chip See Protocol EP155 Live Staining of Cells.
Recommended reagent dilution and incubation time	Dilute 5 mM stock CDFDA solution 1:500 in culture medium. Incubate in the dark at 37°C for 30 minutes.
Representative image	 Image of CDFDA (green) and nuclei staining (blue) indicating hepatocyte polarization, establishment of bile canaliculi structures, and active MRP2 transporters in hepatocytes in the human Liver-Chip (top channel).
More information on vendor site	https://www.thermofisher.com/order/catalog/product/C369?SID=srch-hj-c369