

Protocol for Emulate Organ-Chips:

Live Staining of Reactive Oxygen Species Using CellROX®

April 4, 2019

EP142 v1.0



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Goals:	Key Steps:	Other Required Materials:
Visualize reactive oxygen species (ROS) via fluorescent imaging in live Organ-Chip	 Live staining and fluorescent imaging in Organ-Chips 	 CellROX® Deep Red Reagent (Thermo Scientific[™] 10422) or CellROX® Green Reagent (Thermo Scientific[™] C10444) Cell culture media Fluorescence microscope Optional PBS 4% paraformaldehyde (PFA)

1. Background

CellROX® is a fluorogenic probe for detecting cellular oxidative stress in live cells.

2. Method

Sample type	Live Organ-Chip		
Sample type	See Protocol EP155 Live Staining.		
Recommended reagent dilution and incubation time	Dilute 1:500 in cell culture media. Incubate for 30 minutes at 37°C.		
	Chips can be fixed immediately after staining: 4% PFA for 15 minutes at room temperature.		
Fixative (optional)	The signal remains for up to 2-hours after fixation for reagent C10422, and up to 3-days for reagent C10444.		
	Note: Do not fix cells before CellROX® staining.		
	See Protocol EP137 Fixation and Immunofluorescence (IF) Staining.		
Representative image	Image of the reactive oxygen species stained by CellROX® Deep Red reagent (cyan) in human hepatocytes treated with a compound in the Liver-Chip.		
More information on vendor site	https://www.thermofisher.com/order/catalog/product/C10422		



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3. Organs-Chips in which the staining has been validated

CellROX® Deep Red Reagent (Thermo Scientific[™] C10422) has been validated on Liver-Chips. CellROX® Green Reagent (Thermo Scientific[™] C10444) has been validated on Caco-2 Intestine-Chips. Note: We recommend using the aforementioned reagents for each organ model to achieve best results.

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