



emulate

Protocol for Emulate Organ-Chips:

5-Ethynyl-2'-Deoxyuridine (EdU) Labeling

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EP219 v1.0

TITLE 5-Ethynyl-2'-Deoxyuridine (EdU) Labeling	DOCUMENT EP218	VERSION 1.0
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Goals:	Key Steps:	Other Required Materials:
Assess the actively proliferating epithelial cells in the Emulate Colon Intestine-Chip	<ul style="list-style-type: none"> <li>• Perform the EdU pulse</li> <li>• Fix cells in the chip</li> <li>• Run the reaction</li> <li>• Image the chip</li> </ul>	<ul style="list-style-type: none"> <li>• Click-iT™ EdU Cell Proliferation Kit for Imaging (Thermo Fisher Scientific, Cat. # <a href="#">C10337</a>)</li> <li>• Serum (animal species must match the species that the secondary antibodies were raised in)</li> <li>• TritonX-100</li> <li>• PBS 1X</li> <li>• DAPI for staining nuclei</li> <li>• P0.5–P1000 pipettes and tips</li> </ul>

## Introduction

This protocol uses the Colon Intestine-Chip as a reference point aiming to the quantification of the percentage of actively proliferating epithelial cells, based on the incorporation and subsequent detection of 5-Ethynyl-2'-Deoxyuridine (EdU) in the newly synthesized DNA. The duration of the EdU pulse may differ with a different cell type or Organ-Chip. For the Colon Intestine-Chip it is recommended overnight pulse.

## Method

Sample type	Fixed epithelial cells in the Primary Colon Intestine-Chip. See Emulate Protocol <a href="#">Immunofluorescence</a> for instructions related to fixation of the chips with 4% Paraformaldehyde.
Recommended assay flow rate (Colon Intestine-Chip)	30 µL/h
Recommended EdU concentration and pulse time (Primary Colon Intestine-Chip)	Dilute EdU in the culture medium of epithelial cells at a final concentration of 20 µM. Pulse the top channel, containing the epithelial cells, with EdU 24 h prior to fixing the chip.  Note: Pulse duration may need to be modified to identify proliferating cells with a different length of cell cycle.
Run assay as per manufacture instructions	<a href="https://www.thermofisher.com/order/catalog/product/C10337#/C10337">https://www.thermofisher.com/order/catalog/product/C10337#/C10337</a>  Note: Store components of the kit at 4°C and -20°C immediately upon receipt as per the manufacturer's instructions.

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