emulate

Protocol for Emulate Organ-Chips:

Immunofluorescence Staining—Colon Intestine-Chip

April 8, 2021

EP217 v1.0

Goals:	Key Steps:	Other Required Materials:
Fixing and IF staining of cells in Emulate Colon Intestine-Chip	• Fix cells in the chip • Permeabilizing and blocking for antibody staining • IF staining	• 4% paraformaldehyde • Methanol • TritonX-100 • PBS 1X • Serum (animal species must match the species that the secondary antibodies were raised in) • Primary and secondary antibodies (depending on marker of interest to be stained) • DAPI for staining nuclei • 12-well culture plates • P0.5–P1000 pipettes and tips

Introduction

This is the method we have developed for fixation and immunofluorescence staining for the Colon Intestine-Chip. We realize, however, that users may have their own fixation and staining processes that have been developed for specific cell types, antibodies, or antigens. If users would prefer to use other fixatives, permeabilizing solutions, or blocking buffers, they may do so while following the process outlined below.

Method

Part I — Fixation of cells in Organ-Chips

1. Prepare the workspace of the chemical fume hood prior to beginning your work, ensuring that the space within the hood is organized, free from clutter, and the path of airflow is not blocked. Note: You may be using 4% paraformaldehyde (PFA in PBS) as part of this protocol. PFA is a hazardous chemical. Exposure risk to PFA can be greatly reduced by working in a chemical fume hood and using proper protective equipment for handling.
2. Ensure all chip carriers are labeled and identify the different conditions clearly. Detach chips from Pod™ modules and organize them in petri dishes for handling.
3. Gently wash each channel with 200 µL PBS once.
4. Choose a fixation method based on the epitope of interest, and proceed accordingly:
    a. **4% paraformaldehyde (PFA, in PBS, pH 7.4):** Perfuse 100 µL of 4% PFA to each channel from the inlet, while aspirating the flow through on the outlet side. Incubate for 15 minutes at room temperature.
    b. **Methanol:** Perfuse 100 µL of ice cold methanol to each channel from the inlet, while aspirating the flow through on the outlet side. Incubate for 30 minutes at -20°C.
5. After incubation wash each channel with 200 µL PBS 3 times.

Note: Fixed chips can be stored at 4°C for up to one week in PBS. To ensure channels do not dry up during this period, it is recommended that a humidified chamber is used and sealed with parafilm.

Part II — Permeabilization and Blocking

1. Prepare permeabilization buffer by adding 10% serum (from the animal species the secondary antibodies were raised in) to the solution of 0.1% TritonX-100 in PBS.
2. Perfuse 100 µL of this permeabilizing solution to the channel that contains the desired cell type from the inlet, while aspirating the flow through on the outlet side.
3. Incubate chips for 15 minutes, in the case of membrane target epitopes, or 30 minutes, in the case of intracellular targets, at room temperature.
4. After incubation wash each channel with 200 µL PBS 3 times.
5. Prepare blocking buffer by adding 10% serum (from the animal species the secondary antibodies were raised in) in PBS.
6. Perfuse 100 µL of this blocking solution to the channel that contains the cell type of interest from the inlet, while aspirating the flow through on the outlet side.
7. Incubate for 1 hour at room temperature.
8. After incubation proceed to immunofluorescence staining.

Part III — Immunofluorescence Staining

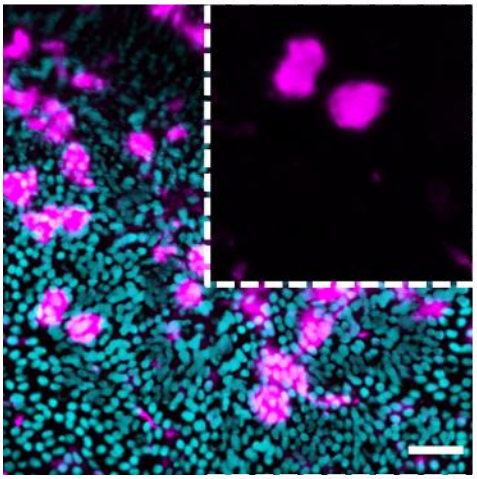
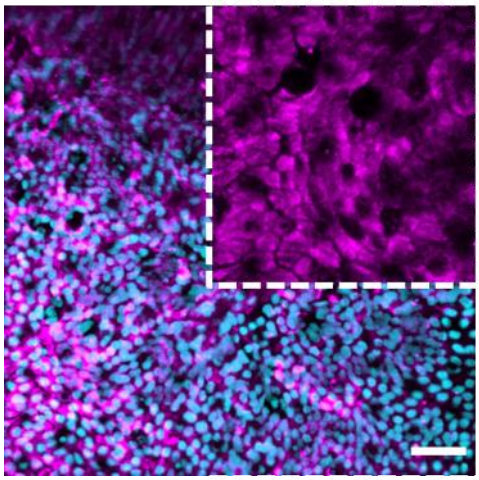
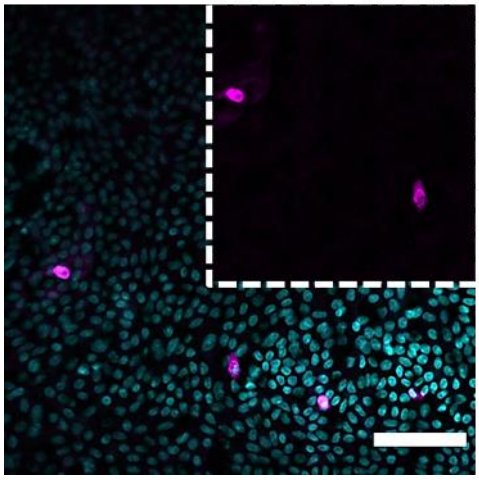
1. Prepare primary antibody solution(s) for each channel by diluting the desired primary antibodies in 5% serum in PBS.
2. The concentration of the antibodies will vary with each antibody used. This must be optimized prior to staining the chips. We summarize the optimal dilutions for some indicative antibodies in the table below.

Target Epitope	Host Species	Vendor	Catalog #	Dilution Ratio	Fixation Method
Mucin 2	ms	Santa Cruz Biotechnology	sc-515032	1: 300	Methanol
Villlin	rb	Abcam	ab130751	1: 100	4% PFA
Chromogranin A	rb	Abcam	ab15160	1: 100	4% PFA
ZO-1	ms	Thermo Fischer	339194	1: 100	4% PFA
E- cadherin	ms	Abcam	ab1416	1: 100	4% PFA
Occludin	ms	Thermo Fischer	33-1500	1:100	4% PFA
Claudin 4	rb	Thermo Fischer	36-4800	1:100	4% PFA
Na^+K^+ATPase	ms	Abcam	ab7671	1:100	4% PFA
SLC26A3 (DRA)	rb	Abcam	ab244452	1:100	4% PFA
SLC9A3 (NHE3)	rb	Novus Biologicals	NBP1-82575	1:100	4% PFA
VE-cadherin	rb	Abcam	ab33168	1:100	Methanol

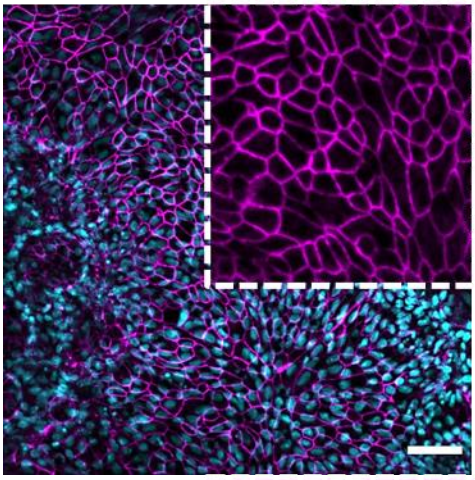
3. After preparing the primary antibody solution(s), add 100 µL (50 µL in the case of half chip) to the channel that contains the desired cell type from the inlet, while aspirating the flow through on the outlet side.

4. Incubate chips overnight at 4°C.
5. After incubation wash each channel with 200 µL PBS 3 times.
6. Prepare secondary antibody solution(s) for each channel by diluting the desired secondary antibodies in 5% serum in PBS.
7. Add 100 µL of the secondary antibody solution(s) (50 µL in the case of half chip) to the channel that contains the desired cell type from the inlet, while aspirating the flow through on the outlet side.
8. Incubate chips for 2 hours at room temperature taking care to protect them from light.
9. After incubation wash each channel with 200 µL PBS 3 times. Optional Step(s):
    a. To stain nuclei, prepare a solution of 50µg/ml DAPI (Invitrogen, Carlsbad, CA, Catalog No: D1306) in PBS.
    b. If nuclei staining is desired, then add 100 µL (50 µL in the case of half chip) to the channel that contains the desired cell type from the inlet, while aspirating the flow through on the outlet side.
    c. Incubate chips for 15 minutes at room temperature, taking care to protect the chips from light.
    d. After incubation wash the channel with 200 µL PBS 3 times.
10. The chips are now ready to image. Refer to Protocol EP126 Fluorescence Imaging for further direction. Indicative maximum projected confocal micrographs, for each of the antibodies stated above, are listed in the table below.

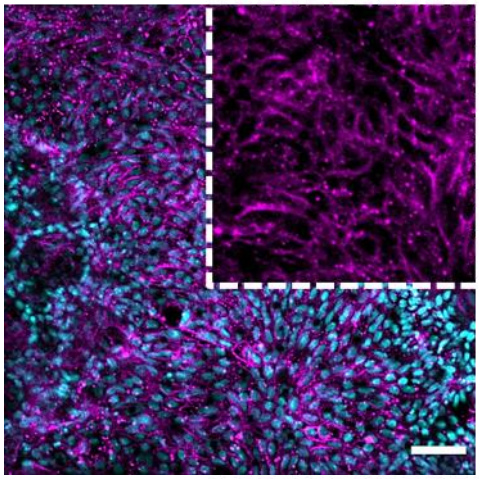
Note: Stained chips can be stored in PBS for up to two weeks at 4°C. To ensure channels do not dry up during this period, it is recommended that a humidified chamber is used and sealed with parafilm

Epitopes	Confocal Micrograph (scale bar 50 µm)
Mucin 2 DAPI	
Villin DAPI	
Chromogranin A DAPI	

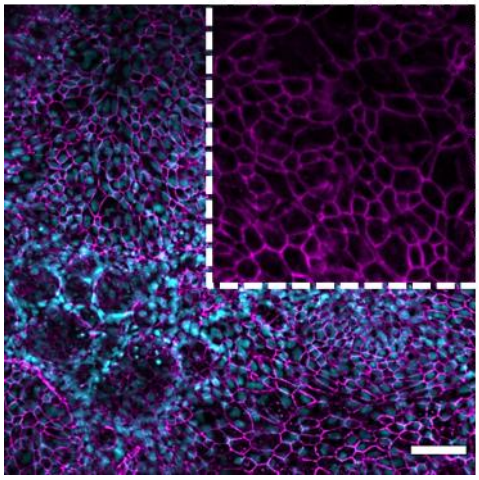
ZO-1
DAPI



E-cadherin
DAPI



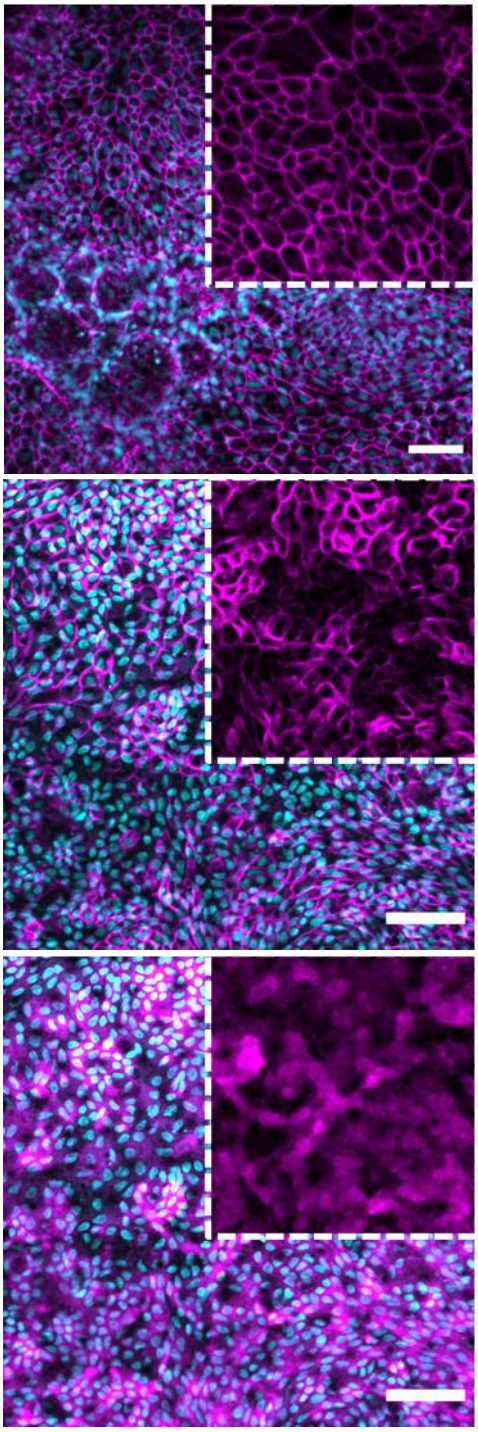
Occludin
DAPI



Claudin 4
DAPI

Na^+K^+ATPase
DAPI

SLC26A3 (DRA)
DAPI



SLC9A3 (NHE3)
DAPI

VE-cadherin
DAPI