Interreg Italia-Österreich

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UNIUD-CCO-SOP-2.0	Author: **M. Codrich** Approved: **G. Tell**	Issued: **15/02/2019** Revised:

INTESTINAL ORGANOIDS IMMUNOFLUORESCENCE ANALYSIS

Purpose

The purpose of SOP 2.0 is to describe how to perform immunofluorescence on intestinal organoids.

Scope

SOP 2.0 is intended to cover all resources, personnel and equipment in the intestinal organoids laboratory.

1. Cell culture media, reagents and solutions

Reagent	Catalogue number and company
PBS without Ca2+, Mg2+ (PBS)	#BE17-512F Lonza
Paraformaldehyde (PFA)	#158127 Sigma-Aldrich
Glycine	#50046 Sigma-Aldrich
Fetal Bovin Serum (FBS)	#S1810-500 BioWest
Bovine Serum Albumin (BSA)	#A2153 Sigma-Aldrich
Tween20	#P1379 Sigma-Aldrich
Triton-X100	#T8787Sigma-Aldrich
NaCl	#S7653 Sigma-Aldrich
Trizma	#T1503 Sigma-Aldrich
poly-L-lysine	# # P0899 Sigma Aldrich
Alexa Fluor- 594 Phalloidin	#A12381 Life technologies
DAPI	#D1306 ThermoFisher
Mowiol 4-88	#475904 Calbiochem
Glycerol	#G5516 Sigma Aldrich
1,4-diazabicyclo-[2,2,2]-octane (DABCO)	#D27802 Sigma Aldrich
Sterile H2O	

2. Equipment

- Pipetaid (SN603280198 Euroclone)
- Dissection tweezers (#11254-20 F.S.T.)
- Confocal microscope (LEICA TCS SP8, Wetzlar, Germany)
- Rocker (PS-M3D Grant-bio)
- Coverslips (#0111530 Paul Marienfeld GmbH & Co.KG)
- Miscroscope slides (#AA00000112E01 Menzel Gläser)

3. Plastics

- 24-multiwell TC-Plate (#ET3024 Euroclone)
- Pipettes (#EPS05N, #EPS10N, #EPS25N Euroclone)
- 10 µl, 200 µl and 1000 µl pipette tips (#ECTD50010RN, #ECTD50200RN, #ECTD51000RN Euroclone)
- 1.5 ml microcentrifuge tubes (#ET3415 Euroclone)
- 50 ml and 15 ml conical tubes (#ET5050B, #ET5015B Euroclone)

4. Antibodies

Primary antibodies (Ab I):

- Lysozyme Dako A009902-2, working dilution 1:200
- OLFM4 Cell Signaling 14369S, working dilution 1:200
- Ki67 Abcam AB92742, working dilution 1:200
- E-Cadherin BD Bioscience 610404, working dilution 1:50

Secondary antibodies (Ab II):

- goat anti-rabbit 488 #711-546-152 Jackson Immunoresearch, working dilution 1:200
- goat anti-mouse 488 #715-546-150 Jackson Immunoresearch, working dilution 1:200

5. Solutions

Mowiol mounting medium:

- Add 2.4 g of Mowiol 4-88 to 6 g of glycerol. Stir to mix. Add 6 mL of H2O and leave for several hours at room temperature. Add 12 mL of 0.2 M Tris-Cl (pH 8.5) and heat to 50°C for 10 min with occasional mixing. After the Mowiol dissolves, clarify by centrifugation at 5000g for 15 min. For fluorescence detection, add DABCO to 2.5% to reduce fading. Aliquot in airtight containers and store at –20°C. Stock is stable at room temperature for several weeks after thawing.

PFA solution:

- 4g of PFA in PBS

Washing Buffer A:

- 0.01M Tris HCl pH 7.4
- 0.15M NaCl
- 0.05% Tween 20

Permeabilization solution (in PBS):

- 0,5% Triton X-100

Blocking solution (in Washing Buffer A):

- 10% FBS
- 1% BSA
- 0.3% Triton X-100

6. Procedure

1. Coat coverslips with poly-L-lysine for 1 h at 37°C.
2. Rinse coverslips well with sterile H2O (three times).
3. Allow coverslips to dry completely and sterilize them under UV light for 1 h.
4. Grow organoids on glass coverslips.
5. Remove medium. Fix with 4% PFA for 20 min at 37°C (500 µl/24-multiwell).
6. Rinse 3 times with PBS for 5 min RT (shaking slowly).
7. Incubate with glycine 0.1M for 10 min RT (shaking slowly).
8. Rinse 3 times with PBS for 5 min RT (shaking slowly).
9. Incubate with PBS + Triton X-100 0,5% for 5 min RT (shaking slowly)

10. Rinse 3 times with PBS for 5 min RT (shaking slowly).
11. Block with 10% FBS + 1% BSA + 0.3% Triton X-100 in Washing Buffer A (Tris HCl pH 7.4 0.01M + NaCl 0.15M + Tween 20 0.05%) for 1h RT (shaking slowly).
12. Incubate Ab I in blocking solution o/n at 4°C.
13. Rinse 3 times with PBS for 5 min RT (shaking slowly).
14. Incubate Ab II in blocking solution for 2 h at RT (shaking slowly).
15. Rinse 3 times with PBS for 5 min RT (shaking slowly).
16. Incubate with Phalloidin 594 200 units/mL for 20 min RT (shaking slowly).
17. Rinse 3 times with PBS for 5 min RT (shaking slowly).
18. Incubate with DAPI 300 nM for 5 min RT (shaking slowly).
19. Rinse 3 times with PBS for 5 min RT (shaking slowly).
20. Rinse with H2O.
21. Clean the coverslip with EtOH. Mount coverslip with a drop of Mowiol mounting medium.
22. Seal coverslip with nail polish to prevent drying and movement under microscope.

See Figure 1

7. Applicable referenceS

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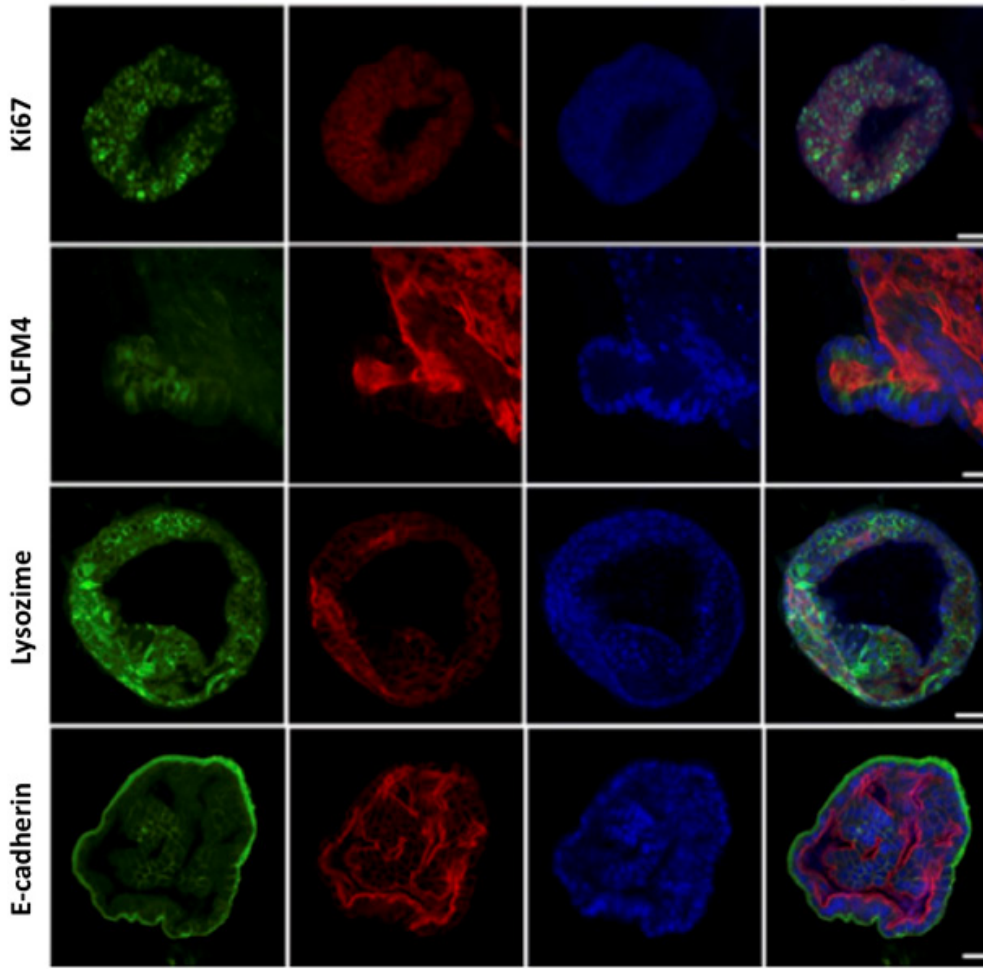

Figure 1 Human intestinal organoids maintain the anatomical characteristic of the tissue of origin. Confocal images of normal undifferentiated intestinal organoids (passage 5, cultured in medium +Wnt3a). Immunofluorescence (IF) was performed with anti-Ki67, anti-OLFM4, anti-Lysozime and anti-E-chaderin antibodies (green). F-actin was marked by Phalloidin594 (red) and nuclei were marked by DAPI (blue). Magnification 25X. Scale bar 25 µm (OLFM4 and E-chaderin) and 50 µm (Ki67 and Lysozime).