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LNCIB
SOP –LCO-2.0

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LUNG ORGANOIDS IMMUNOFLUORESCENCE IN MATRIGEL

Purpose

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

Scope

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

Introduction

Molecular characterization of lung organoids can be performed using different techniques. Here the procedure for immunofluorescence analysis of human lung organoids using Anti-Mucin 5ac antibodies and Phalloidin is described. In lung tissue, Mucin 5ac is specifically expressed in mucus secreted goblet cells; fluorescently-labelled phalloidin selectively stain F-actin filaments.

1. Reagents

- Paraformaldehyde (Sigma-Aldrich, cat. no. 158127)! !CAUTION Paraformaldehyde contains formaldehyde, which can cause cancer; handle it using appropriate safety gear.
- PBS-1x (Life Technologies, cat. no. 14190-094)
- Glycine (Sigma-Aldrich, cat. no.50046)
- Bovine Serum Albumin, BSA (Sigma-Aldrich, cat. no.A2153)
- Fetal Bovine Serum, FBS (Euroclone, cat. no. ECS0180L)
- Trizma (Sigma-Aldrich, cat. no.T1503)

- Hydrochloric acid, HCl (Sigma-Aldrich, cat. no. H1758)
- Sodium Chloride, NaCl (Sigma-Aldrich, cat. no.S7653)
- Tween20 (Sigma-Aldrich, cat. no.P1379)
- Poly-L-lysine (Sigma-Aldrich, cat. no.P4707)
- Triton-X100 (Sigma-Aldrich, cat. no.T8787)
- Normal donkey serum (Sigma-Aldrich, cat. no. D9663-10ml)
- DAPI (Sigma, cat. no. D9542)
- Vectashield mounting medium without DAPI (Vector, cat. no. H1000)
- Anti-Mucin 5AC EA primary antibody diluted 1:20 in blocking solution (Thermo Scientific cat.no. MA512178)
- Anti-mouse Alexa fluor 488 diluted 1:500 in blocking solution (Invitrogen cat. no. A32723)
- Phalloidin594 (Life Technologies, cat. no.A12381)
- Cover glasses circles (Life Technologies, cat. no. 12-545-80)
- Syringes filters pes 0,22µm (Euroclone, cat. no. EPSPE2230)

2. Reagent setup

- Blocking solution :10% FBS; 1% BSA; 0.3% Triton X-100 in 0.01M TrisHCl pH 7.4, 0.15M NaCl, 0.05% Tween 20

3. Reagent setup

Stock solution:

- Prepare stock solution by dissolving 100mg poly-L-lysine in 100ml water. Filter sterilize the solution by passing through a 0.22µm filter. Store at-20˚C.

Coating Coverslips:

- Working solution: 50 µg/ml (1:20 dilution of stock).
  - Sterilize the coverslips in 95% ethanol and dry before coating.
  - Place the coverslips in a 24 well plate and coat cover glasses with 80µl of poly-L-lysine (50µg/ml dissolved in sterile H2O) for 1 h at 37˚C.
  - Rinse coverslips with 1ml of sterile H2O (at least three times).

4. Equipment

- High-speed centrifuge (Eppendorf centrifuge 5810R)
- Pipette aid, serological pipettes (Euroclone, cat. no. EPS05N; EPS10N)
- Pipettes
- Pipette tips
- Conical centrifuge tubes (Euroclone, cat. no. ET5015B; ET5050B)
- Microscopes slides Superfrost® Plus (Thermo Scientific, cat. no. J1800AMNZ)
- Microcentrifuge tubes (Euroclone, cat. no. ET3415)
- Cover glasses circles (Life Technologies, cat no. 12-545-80)
- Confocal fluorescence microscope

5. Procedure

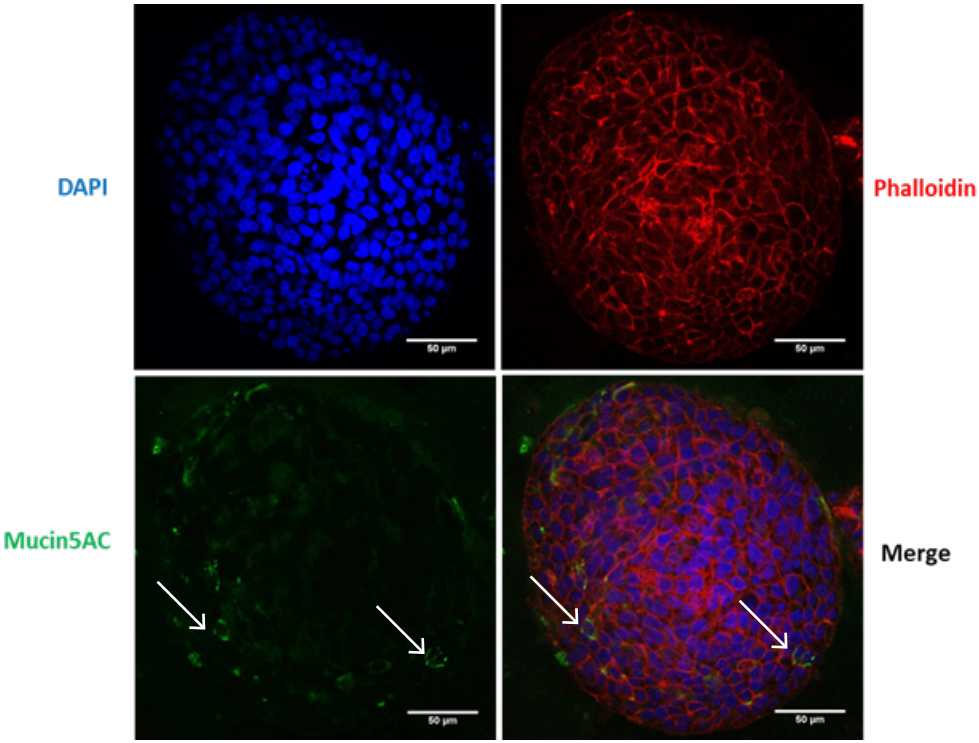
- Grow organoids in matrigel on glass coverslips, for 5-7 days by following procedures described in LNCIB SOP –LCO-1.0.
- Wash organoids with 1 ml of 1x PBS for 5 min RT (shaking slowly).
- Aspirate off medium.
- Fix organoids on coverslip with 4% Paraformaldehyde for 20 min at 37°C (500 µl/24-well).
- Wash organoids with 1 ml of 1x PBS for 5 min RT (shaking slowly). Repeat the procedure 3 times.
- Incubate with 1 ml of 0.1M Glycine for 10 min RT (shaking slowly).
- Wash organoids with 1 ml of 1x PBS for 5 min RT (shaking slowly). Repeat the procedure 3 times.
- Incubate with 1 ml of 1x PBS, 0,3% Triton X-100 for 5 min RT (shaking slowly).
- Wash organoids with 1 ml of 1x PBS for 5 min RT (shaking slowly). Repeat the procedure 3 times.
- Incubate organoids with 1ml of blocking solution for 1h at RT

(shaking slowly).

- Incubate sample with 200 µl of Anti- Mucin 5AC EA primary antibody diluted 1:20 in blocking solution o/n at 4°C.
- Wash organoids with 1 ml of 1x PBS for 5 min RT (shaking slowly). Repeat the procedure 3 times.
- Incubate sample with 200 µl of Anti-mouse secondary antibody diluted 1.500 in blocking solution for 2 hours at RT (shaking slowly).
- Wash organoids with 1 ml of 1x PBS for 5 min RT (shaking slowly). Repeat the procedure 3 times.
- Incubate with 500 µl of DAPI (1µg/ml in H2O) for 5 min at RT (shaking slowly).
- Wash organoids with 1 ml of 1x PBS for 5 min RT (shaking slowly). Repeat the procedure 3 times.
- Mount coverslip in a drop of mounting medium.

Note: if you use Phalloidin, incubate Phalloidin594 (1:500 in blocking buffer) for 20min RT (shaking slowly) after secondary incubation and proceed with washing. (See ***figure1***).

Figure 1. Immunofluorescence of lung tumor organoids. Blue: DAPI (nuclei), red: Phalloidin (F-actin) and green: Mucin5ac (secretory goblet cells). Arrows indicate the intracellular localization of Mucin5A.



6. Applicable references to LNCIB SOPs

LNCIB SOP –LCO-2.0, LNCIB SOP –LCO-3.0

7. Applicable references

1. Long-term expanding human airway organoids for disease modelling. Normal Sachs et al. EMBO J; 38(4); 2019. doi: 10.15252/embj.2018100300.

2. Differentiated human airway organoids to assess infectivity of emerging influenza virus. Zhou J. et al; Proc Natl Acad Sci U S A; 115(26), 20182018. doi: 10.1073/pnas.1806308115