

Feb 24, 2022

Immunofluorescent staining of formalin-fixed OCT-embedded human lung tissue

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1

dx.doi.org/10.17504/protocols.io.b2jncme

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This protocol describes steps for immunofluorescent staining of formalin-fixed paraffin-embedded human lung tissue.

DOI

dx.doi.org/10.17504/protocols.io.b2jncme

Justinn Barr, Jamie Verheyden, Xin Sun 2022. Immunofluorescent staining of formalin-fixed OCT-embedded human lung tissue. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.b2jncme>



protocol ,

Dec 02, 2021

Feb 24, 2022

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Primary Antibodies

A	B	C	D	E	F
Antibody	Dilution	Company	Catalog Number	Lot number	RRID
Neuron-specific beta-III Tubulin Biotin MAb (Clone TuJ-1)	1:100	R&D Systems	BAM1195	HVS0215121	AB_356859
Human CD31/PECAM-1 MAb (Clone 9G11)	1:100	R&D Systems	BBA7		AB_356960
Anti-Tyrosine Hydroxylase Antibody	1:100	MilliporeSigma	AB152		AB_390204
Anti-Synaptophysin (Neuroendocrine Marker) Ab-4 Polyclonal Antibody, Unconjugated	1:100	Fisher Scientific	RB-1461-P0	1461P1603G	AB_60082
Monoclonal Anti-Actin, alpha-Smooth Muscle - Cy3	1:100	Sigma-Aldrich	C6198	086M4829V	AB_476856
Anti-VACHT (rabbit, polyclonal)	1:100	Synaptic Systems	139 103		AB_887864
Anti-TAGLN/Transgelin (SM22)	1:100	Abcam	ab14106	GR274712-1	AB_443021
Anti-Calcitonin Gene Related Peptide (CGRP) antibody produced in rabbit	1:100	Sigma-Aldrich	C8198	066M4836V	AB_259091
Anti-P2X3 Receptor	1:100	Sigma-Aldrich	AB5895		AB_177544
Anti-VGLUT 2 antibody	1:100	Synaptic Systems	135 403		AB_887883
Anti-PGP9.5 [13C4 / I3C4]	1:100	Abcam	ab8189		AB_306343

Primary Antibodies

Secondary Antibodies

A	B	C	D	E	F
Antibody	Dilution	Company	Catalog Number	Lot number	RRID
Cy3-AffiniPure Goat Anti-Mouse IgG	1:300	Jackson ImmunoResearch Labs	115-165-003	129939	AB_2338680
Fluorescein (FITC)-AffiniPure Goat Anti-Rabbit IgG (H+L)	1:300	Jackson ImmunoResearch Labs	111-095-144		AB_2337978

Secondary Antibodies

- 1 2X5 min wash in Phosphate-buffered saline with Tween (PBSTW) in a slide holder on a flat rotator.


2 **Blocking:**

- 2.1 Mix up 5% serum ( 50 μ L serum /  1 mL of PBSTW)

Normal goat serum in most cases.

- 2.2 Remove old PBSTW from the sample and add block.

- 2.3 Cover with Hybrislip.

- 2.4 Let sit in a humidified chamber at  **Room temperature** for ≥ 1 hour.

Primary antibody:

3

- 3.1 Mix up more of the 5% serum.
- 3.2 Add antibody to the serum at the desired concentration.
- 3.3 Take the humidified chamber to a cold room.
- 3.4 Add primary antibody in the cold room.
- 3.5 Cover with Hybrislip.
- 3.6 Seal the humidified chamber.

4 *On Day 2:*

Wash slides 3X10 min at  **Room temperature** in slide holder on a flat rotator.

5 *Secondary antibody:*

- 5.1 Mix up 5% serum.
- 5.2 Add secondary antibody to the serum at the desired concentration.

5.3 Add antibody mix to the slides.

5.4 Let it sit in a humidified chamber **EITHER** at **8 Room temperature** for 1 hour **OR** in the cold room overnight.

5.5 Cover with Hybrislip.

6 Wash:

6.1 1x10 min in PBSTW with DAPI.

6.2 2x10 min in PBSTW.

7 Mount the slides in VectaShield and seal with nail polish.