

Oct 07, 2023

Yale Murine TMC - Immunofluorescence Protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5xenng1b/v1>

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Hester Doyle

Yale SenNet murine TMC

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Protocol Citation: Yun-Hee Youm, Yufeng Liu, Vishwa Deep Dixit 2023. Yale Murine TMC - Immunofluorescence Protocol. protocols.io <https://dx.doi.org/10.17504/protocols.io.8epv5xenng1b/v1>

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Protocol status: Working

We use this protocol and it's working

Created: October 07, 2023



Last Modified: October 07, 2023

Protocol Integer ID: 88929

Keywords: immunofluorescence protocol immunofluorescence, immunofluorescence, yale murine tmc, staining, mouse

Abstract

Immunofluorescence staining of mouse tissues

Immunofluorescence Staining

- 1 Take out the slides to a slide holder and warm slides on a 37°C heater for 5-10 min. Grouping: usually maximum two antibodies (with secondary antibody red + green) + nuclear staining for one slide. Set negative control for each secondary antibody to detect any non-specific background.
- 2 Label slides with pencil and circle section with Pap Pen (hydrophobic marker). Move the slides into a humidified chamber. **Slides should remain in humidified chamber for all the following incubation steps.**
- 3 Fixation: Add one or two drops of 4% paraformaldehyde in PBS on the tissue for 15-30 min.
- 4 Wash with 0.5% Tween 20 in PBS (PBST) , 10 min x 2.
- 5 Block with Blocking buffer: 10% host serum + 2% FBS in 0.5% PBST. Incubate 1 hour at room temperature or 4°C overnight.
- 6 Incubate with primary antibody: 1-10 µg/mL of purified antibody or 1:100 to 1:1000 dilution of anti-serum in 2% host serum + 2% FBS 0.5% PBST buffer overnight at 4°C. Normally start with a dilution at 1:100. For color-conjugated FC antibodies used in immunofluorescence, start at 1:50.
- 7 Wash with 0.5% PBST, 10 min x 2.
- 8 Incubate with secondary antibody: 1-10 µg/mL of purified antibody or 1:100-1:1000 of anti-serum in Blocking buffer (normally start at 1:200). Incubate 1 h at room temperature (**AVOID LIGHT**).
- 9 Wash with PBST, 10 min x 3.
- 10 Incubate the slides in 0.1 µg/mL DAPI for 5 min.
- 11 Wash with PBST twice before mounting.
- 12 Clean the slides.



- 13 Mount the slides with coverslips using anti-fade mounting medium.
- 14 Seal the edges of the coverslip with nail polish and let it dry.