Yale Murine TMC - Immunofluorescence Protocol

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

Immunofluorescence staining of mouse tissues

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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% parformaldehyde 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.