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Free floating Immunofluorescence protocol for mouse brain sections V.2

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We use this protocol and it's working

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Abstract

Free floating Immunofluorescence protocol with multiplexing labelling for mouse brain sections

Day 1

5h

1 Pour sections into a well insert in a 6-well plate with 1X PBS to separate storage solution from sections. Change 1X PBS before step 3.

2 Remove samples from freezer and equilibrate at room temperature (RT) for 10 - 20 minutes.

3 Wash once with 1X PBS for 20 minutes and once with 1X PBS for 10 minutes on an orbital shaker using moderate speed at RT.

4 Incubate the sections in 0.01M sodium citrate buffer (pH 6) at 85°C for 30 minutes with the lid on and place a ceramic in the bottom of the bath and a top lid for the bath to keep temperature stable. Let it cool to RT temp and dab it on tissue paper.

5 Rinse the sections 3x 10 mins in 1X PBS.

6 While sections are washing, prepare 7 mL (per sample) of a blocking-permeabilizing solution consisting of 1X PBS with

7 3% Triton X-100 and 3% normal serum (e.g., normal horse serum) in 6 well plate. Move to humid chamber and block sections for 1 hour at RT on orbital shaker, using low speed.

8 Prepare 400 µl per sample of primary antibody solution consisting of selected primary antibody (diluted appropriately) in 1X PBS with 0.3% Triton X-100 and 1% normal serum (see Step 4.4 Note). NOTE: Multiple primary antibodies may be used (generated in different host species).

9 Place ceramic plate on rocker using low speed and incubate 2 nights 4°C in a humid chamber with zip lock bag.

Day 2

5h

10 The following day, pour sections into a well insert in a 6-well plate to separate sections from primary antibody solution. NOTE: Antibody solution can be collected and reused; add 0.02% (w/v) sodium-azide to inhibit microbial growth.

11 Wash sections 3X 10 minutes with 1X PBS at RT

12 Prepare 1 ml per sample of secondary antibody solution consisting of appropriate secondary antibody (diluted accordingly) in 1X PBS with 0.3% Triton X-100 and 1% normal serum (avoid light).

13 Transfer sections into a ceramic plate. Incubate for 2 hours at RT on orbital shaker using low speed (avoid light).

14 Pour sections into a well insert in a 6-well plate to separate sections from secondary antibody solution.

15 Wash 3x 5 minutes with 1X PBS at RT (avoid light).

Mounting

2h

16 Pour sections petri dish filled with 1X PBS.

17 Submerge a glass slide into the 1X PBS and use a fine paintbrush to spread open the sections onto the slide.

18 Gently unfold any wrinkles with correct orientation from rostral to caudal, top left to right follow by bottom left to right. Repeat until all sections are mounted onto the slide(s).

Cover slipping

30m

19 After sections are dried onto the slide(s), about 10-15 minutes at RT or until sections look opaque (remember to shield slides from light), apply an appropriate aqueous mounting medium (non-hardening). Antifading is preferred if using a fluorescent conjugated secondary antibody. NOTE: Fluorescence quality may be lesser when using a hardening mounting medium, but slides should last longer.

20 Using tweezers, place a coverslip on top of the medium. Cover with filter paper and press down firmly to remove excess mounting medium (Daiko GM30411-2). NOTE: If using a non-hardening mount, paint the edges of the coverslipped slide with clear nail polish to seal.

21 Store in a dark slide box at 4°C.