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human alpha-synuclein and aggregated alpha-synuclein immunofluorescence staining

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We use this protocol and it's working

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Abstract

This protocol is designed for human alpha-synuclein staining using the MJFR1-Alexa 488 (RRID:AB_2537217) antibody and either anti-SynO2 (RRID:AB_2632701) or MJFR-14-6-4-2 (RRID:AB_2714215) antibodies. Tissue stained with this protocol include 35 µm free-floating mouse brain sections. All tissue was from mice perfused with 4% PFA.

Day 1

10m

1 Rinse brain slices (35µ) in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) 3X 00:10:00 — 30m

2 Quench brain slices in a solution containing 3%H_2O_2 and 10% Methanol in TBS 00:20:00 — 20m

3 Rinse brain slices in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) 3X 00:10:00 — 30m

4 Incubate in blocking buffer (5% donkey serum, 2% BSA, 0.5% triton X-100 in TBS) 01:00:00 hour at room temperature — 1h

5 Incubate in mouse on mouse blocking buffer (MKB-2213; Vector Lab) 00:20:00 at room temperature — 20m

6 Rinse brain slices in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) 00:00:10 — 10s

7 Incubate with anti-SynO2 (1:1000; RRID:AB_2632701) or anti-MJFR-14-6-4-2 (1:20000; RRID:AB_2714215) dilution in 50% blocking solution 48:00:00 hour at 4°C — 2d

8 Rinse brain slices in TBS-T (TBS+ 0.25% Triton X-100)) 3X 00:10:00 — 30m

9 Incubate in fluorescent secondary antibody at 1:200 dilution in 50% blocking buffer 01:00:00 r at room temperature. — 1h

10 Rinse brain slices in TBS-T (TBS+ 0.25% Triton X-100) 3X 00:10:00 — 30m

11 Incubate with rabbit anti-hASYN(1:400; RRID:AB_2537217, MJFR1-Alexa 488) 24:00:00 at 4°C in 50% blocking buffer — 1d

12 Rinse brain slices in TBS-T (TBS+ 0.25% Triton X-100) 2x 00:10:00 — 20m

13 Rinse brain slices in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) 00:10:00 10m

14 Mount free-floating sections on SuperFrost+ slides (if staining free-floating tissue) and let dry at room temperature for 15 minutes.

15 Coverslip with fluorescent mounting medium and #1.5 coverslips. Outline coverslip with clear nail polish and store at 4oC .