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human alpha-synuclein and aggregated alpha-synuclein immunofluorescence staining

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Protocol status: Working

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 μ m free-floating mouse brain sections. All tissue was from mice perfused with 4% PFA.



Day 1

10m

- 1 Rinse brain slices (35 μ) 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% H₂O₂ 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
- 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 .

10m