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SOD2 and human alpha-synuclein immunofluorescence staining



Forked from [human alpha-synuclein and aggregated alpha-synuclein immunofluorescence staining](#)

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Pietro La Vitola¹

¹German Center for Neurodegenerative Diseases (DZNE)



Pietro La Vitola

German Center for Neurodegenerative Diseases (DZNE), Alignin...

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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 SOD2 and human alpha-synuclein staining using the anti-SOD2 (RRID:AB_2636921) and MJFR1-Alexa 488 (RRID:AB_2537217) 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 (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 with anti-SOD2 (1:1000; Cell Signaling, RRID:AB_2636921) in 50% blocking solution 24:00:00 hour at 4°C 1d
- 6 Rinse brain slices in TBS-T (TBS+ 0.25% Triton X-100) 3X 00:10:00 30m
- 7 Incubate in fluorescent secondary antibody at 1:200 dilution in 50% blocking buffer
 01:00:00 r at room temperature. 1h
- 8 Rinse brain slices in TBS-T (TBS+ 0.25% Triton X-100) 3X 00:10:00 30m
- 9 Incubate with rabbit anti-hASYN(1:400; RRID:AB_2537217, MJFR1-Alexa 488)
 24:00:00 at 4°C in 50% blocking buffer 1d
- 10 Rinse brain slices in TBS-T (TBS+ 0.25% Triton X-100) 2x 00:10:00 20m
- 11 Rinse brain slices in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) 00:10:00 10m
- 12 Mount free-floating sections on SuperFrost+ slides (if staining free-floating tissue) and let dry at room temperature for 15 minutes.



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