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Fluorescent Immunolabelling for endogenous mouse LRRK2

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

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Abstract

This protocol is used to label endogenous LRRK2 in mouse.
It has been edited from West et al., 2014
Protocol optimized for fresh fixed brain tissue sectioned at 40 um.

Materials

Primary Antibody anti-LRRK2 c41-2 **Abcam Catalog #**ab133474

DAPI **Thermo Fisher Scientific Catalog #**D1306

Donkey serum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S30-100ML

Solutions:

Tris Buffer Solution TBS - 2L
- 50mM Tris pH 7.5 – 100 mL of 1M stock
- 150 mM NaCl – 17.53 g
- Distilled Water – 1.9 L

1 Select brain slices, from fresh, unfrozen, fixed tissue - slices are 40um thick

2 Wash 3 times for 00:10:00 in TBS 10m

3 Sections were “quenched” in 100% Methanol for 00:15:00 at 4 °C on shaker. 15m

4 Wash 3 times for 00:10:00 in TBS 10m

5 Incubate the slices with Sodium Citrate – 10 mM Sodium Citrate pH 6.0 w/0.05% Tween for 00:30:00 at 37 °C on shaker. 30m

6 Wash 3 times for 00:10:00 in TBS 10m

7 During the washes, prepare the blocking solution : 5% normal donkey serum (matched to secondary AB) in TBS with 0.3% Triton

8 Incubate the slides with blocking solution for 01:00:00 at 4 °C on shaker. 1h

9 Wash 3 times for 00:10:00 in TBS 10m

10 During the washes, prepare the primary antibody solution : 5% normal donkey serum in TBS with NO TRITON + Primary Antibody anti-LRRK2 (1:500 or 1:1000)

11 Incubate at 4 °C on shaker for 24:00:00 48:00:00 3d

12 Wash 3 times for 00:10:00 in TBS 10m

13 During the washes, prepare the secondary antibody solution : 5% normal donkey serum in TBS with NO TRITON + Alexa Fluor secondary AB (1:1000)

14 Incubate at 4 °C on shaker for 18:00:00 — 18h

15 Wash 2 times for 00:05:00 in TBS — 5m

16 During the washes, prepare the DAPI solution : DAPI (1mg/ml): 1:10,000, in TBS

17 Incubate the slides with the DAPI solution for 00:10:00 — 10m

18 Wash 3 times for 00:10:00 in TBS — 10m

19 Mount sections on superfrost + slides and coverslip with Invitrogen Prolong Antifade reagent.