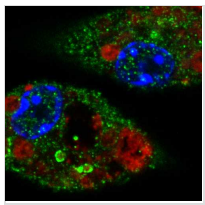

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LRRK2 Immunofluorescent staining

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ABSTRACT

Protocol for immunofluorescent staining for LRRK2 in cultured cells using the MJFF2 (c41-2) antibody.

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MATERIALS TEXT

Reagents:

- PBS, pH 7.4: #14190250, ThermoFisher Scientific
- 4 % (v/v) PFA/PBS: Dilute 16 % Paraformaldehyde Aqueous Solution (#15710, Electron Microscopy Sciences) to 4 % in PBS
- Ice-cold Methanol (pre-chill at - 20 °C before use)
- Blocking and antibody dilution buffer: 5 % (v/v) FCS in PBS
- anti-LRRK2 antibody: MJFF2 (c41-2), ab133474, Abcam
- anti-rabbit-Alexa Fluor™ 488: #A-11034, ThermoFisher Scientific or similar
- DAPI staining solution: 300 nM DAPI in PBS (#D1306, ThermoFisher Scientific or similar)
- Mounting medium: DAKO Fluorescence Mounting medium, # S3023, Agilent or similar

Equipment:

- Coverslips #1.5 (eg 631-0150, VWR) and slides (eg SuperFrost Plus™, J1800AMNZ, Epredia)

OR

- Optical cell culture plate (eg PhenoPlate 96-well, 6055302, PerkinElmer)
- a fine pair of tweezers if using coverslips (eg Artis tweezer, style 5-SA, Z742676-1EA, Sigma-Aldrich)

1 Culture cells as usual on cover slips or in a plate suitable for imaging.

If using coverslips, we recommend Ø13 mm for cells cultured in 24-well plates.

The MJFF2 (c41-2) antibody only detects concentrated LRRK2. Therefore, controls should be included during the sample preparation to ensure signal specificity. We recommend including a LRRK2 KO or knock down control and a positive control such as 30 min treatment with 1mM LLOMe (H-Leu-Leu-OMe•HBr, 4000725.0001, Bachem) or 50 µM chloroquine (C6628-25G, Sigma-Aldrich).

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15m

2 Fix cells in 4 % PFA/PBS at **4 °C** for **00:15:00** .

3

Optional: at this step, coverslips or plates can be stored at 4 °C in PBS. Never allow samples to try out.

10m

4 Permeabilise the plasma membrane by incubating samples in ice-cold MeOH for **00:10:00** . This can be done on the bench or the whole plate can be put into the -20 °C freezer. Make sure that samples are always submerged.

5

Wash samples once in PBS

20m

6

Incubate samples in blocking buffer for **00:20:00** at **Room temperature**

1h

7

Incubate samples in primary antibody solution (MJFF2 (c41-2) antibody diluted 1:100 in blocking buffer) for **01:00:00** at **Room temperature**

> At this step, additional primary antibodies can be added to assess LRRK2 localisation. For lysosomal location in mouse samples, we recommend anti-LAMP1 (#1D4B, Developmental Studies Hybridoma Bank) used at 1:100 dilution.
>
> Always control for cross-reactivity.

8

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Wash samples three times in PBS

9 45m

- Incubate samples in secondary antibody solution (eg anti-rabbit-Alexa Fluor™ 488 diluted 1:800 in blocking buffer) for **00:45:00** at **Room temperature** in the dark

If additional primary antibodies were used, also include additional secondary antibodies here (eg anti-rat-Alexa Fluor™ 657).

10

Wash samples twice in PBS

11 10m

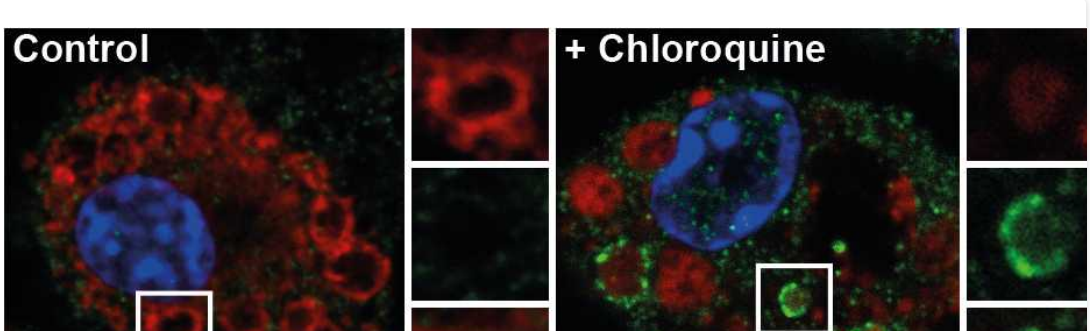
Incubate sample in DAPI staining solution for **00:10:00** at **Room temperature** in the dark

12

Wash samples twice in PBS

13

- Mount coverslips onto slides using a mounting medium of choice (eg DAKO Fluorescence Mounting medium) and let try at RT in the dark.
- If using well-plates, add PBS to plates.
- Keep samples in the dark and store them at 4 °C for short-term storage.



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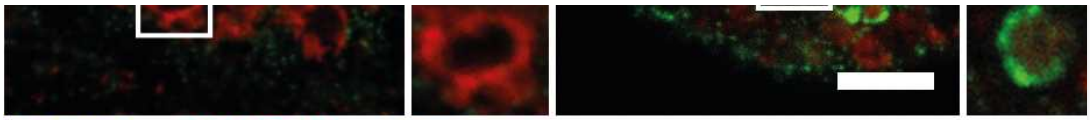

Murine bone marrow-derived macrophages were treated with 50 µM chloroquine for 30 min and stained for LRRK2 according to the above protocol. LRRK2 accumulates at the lysosomal membrane i response to chloroquine but is not visible in untreated cells using this staining method.

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