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🌐 Staggered PFF and Cytokine Treatment in DA Neurons for Confocal, Western Blot, and EM Analysis V.2

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We use this protocol and it's working



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Abstract

This protocol describes the staggered treatment of differentiated dopaminergic (DA) neurons with pre-formed α -synuclein fibrils (PFFs) and either IFN- γ or IL-1 β , optimized to promote inclusion formation while minimizing neuronal toxicity. An alternative 'dual hit' treatment protocol adapted from Bayati et al. is also provided for evaluating interventions such as Parkin activation.

Guidelines

The staggered treatment improves neuronal survival and inclusion formation. The dual hit approach further incorporates Parkin activator treatment post IFN- γ exposure to assess neuroprotective interventions. The 10-day version allows efficient modeling of inclusion pathology with reduced toxicity.

Materials

Differentiated DA neurons on 15-mm coverslips, PFFs (prepared as described in the manuscript), IFN- γ (Thermo Fisher Scientific, PHC4033 or Gibco, 300-02-500UG), IL-1 β (Thermo Fisher Scientific, RIL1BI), Parkin Activator AH231 (4 μ M final concentration), DMSO (vehicle control), BrainPhys neuronal media (STEMCELL Technologies, 05790), PBS

Standard 14-Day PFF and IFN- γ Treatment

- 1 Administer PFFs at 1 $\mu\text{g/ml}$ in BrainPhys media. Incubate for 48 h.
- 2 Replace with fresh BrainPhys media. Incubate for 72 h.
- 3 Replace media with BrainPhys supplemented with 0.2 $\mu\text{g/ml}$ IFN- γ or PBS control. Incubate for 24 h.
- 4 Replace with fresh BrainPhys media. Collect samples at desired time points:
 - 4.1 6-day neurons: immediately after fresh media change.
 - 4.2 7-day neurons: 24 h later.
 - 4.3 10-day neurons: 72 h after 7-day collection.
 - 4.4 14-day neurons: 8 days after media change on Day 6.

Accelerated 2-Day PFF and IFN- γ Treatment

- 5 Administer PFFs at 1 $\mu\text{g/ml}$ in BrainPhys media. Incubate for 24 h.
- 6 Add 0.2 $\mu\text{g/ml}$ IFN- γ (or PBS control) directly into the same media. Continue incubation for another 24 h.
- 7 Collect cells at 48 h total treatment time.

Alternative Dual Hit Treatment (Adapted from Bayati et al.)



- 8 Administer PFFs at 1 µg/ml in BrainPhys media. Incubate for 48 h.
- 9 Replace with fresh BrainPhys media. Incubate for 72 h.
- 10 Replace media with BrainPhys supplemented with 0.2 µg/ml IFN-γ (Gibco, 300-02-500UG). Incubate for 48 h.
- 11 Following IFN-γ treatment, replace with media containing either 4 µM Parkin Activator (AH231) or DMSO vehicle control. Incubate for 36 h.
- 12 Replace with fresh BrainPhys media. Incubate for 36 h.
- 13 Collect cells for fixation, imaging, quantification, or Western blotting at Day 10 post-initial PFF treatment.