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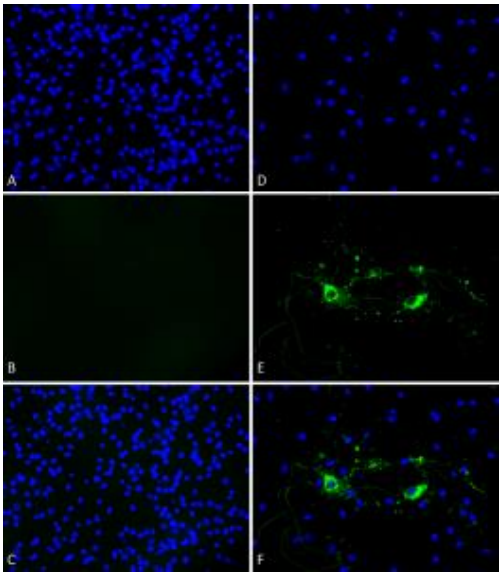
Alpha Synuclein Protocols – Cell Culture Assay

Protocol

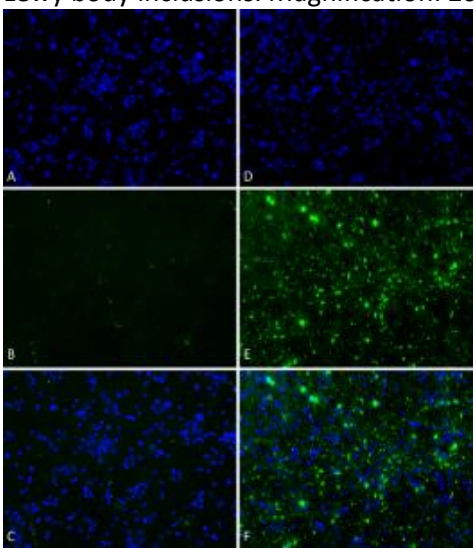
The following protocol was used to generate the ICC images of neurons treated with active and control alpha synuclein preformed fibrils (PFFs). Primary rat hippocampal neurons show lewy body inclusion formation when treated with active alpha synuclein PFFs but not when treated with control alpha synuclein PFFs.

1. Hippocampal neurons harvested from postnatal day 1 Sprague Dawley rat pups and plated at 80,000 cells per well on a poly-D-lysine and laminin coated plate.
2. Preformed fibrils were sonicated for 1 hour in a waterbath sonicator immediately prior to treatment.
3. Neurons were treated with preformed fibrils at 4 µg/mL. Control wells with vehicle were maintained.
4. All wells underwent a 50% media change after 7 days.
5. All wells were fixed with 4% formaldehyde for 20 minutes 7 days after the media change (day-in-vitro 14 after treatment).
6. The formaldehyde was washed off with three washes of filtered 10 mM phosphate buffered saline (10 minutes each).
7. Cells were then blocked with 1:1 PBS:LiCOR Odyssey Block (LiCOR, 927-40010) and 30 mL/mL of 0.1% triton-X 100 for 30 mins.
8. After 30 mins, primary antibody diluted in 1:1 PBS:LiCOR Odyssey Block (with 30 mL/mL of 0.1% triton-X 100) were added to the wells and incubated overnight at 4 degrees.
9. The next day, the primary antibody solution was washed off with three washes of filtered 10 mM phosphate buffered saline (10 minutes each).
10. The secondary antibody was diluted in 1:1 PBS:LiCOR Odyssey Block (with 30 mL/mL of 0.1% triton-X 100) and added to the wells for 60 mins in the dark.
11. After 60 mins, the secondary antibody solution was washed off with three washes of filtered 10 mM phosphate buffered saline (10 minutes each).
12. The plate was then imaged on an epifluorescent microscope (Olympus IX73, B&B Microscopes) at 20X objective.
13. All images were captured at the same fixed scaling and same exposure times.

Primary antibody: Anti-pSer129
Counterstain: Hoechst reagent
Counterstain dilution: 1:3000
Counterstain incubation time: 1 hr



Primary rat hippocampal neurons (DIV16) show lewy body inclusion formation and loss of cells when treated with active mouse Alpha Synuclein Protein Preformed Fibrils (SPR-324) at 4 µg/ml (D-F) on DVI2, but not when treated with a control (A-C). Tissue: Primary hippocampal neurons. Species: Sprague-Dawley rat. Fixation: 3% formaldehyde from PFA for 20 min. Blocker: 1:1 PBS:LiCOR Odyssey Block (LiCOR, 927-40010) and 30 mL/mL of 0.1% triton-X 100 for 30 min. Primary Antibody: Mouse anti-pSer129 Antibody (1:1000) and Rabbit anti-pSer129 (1:800) for 24 hours at 4°C. Secondary Antibody: ATTO 546 Donkey Anti-Mouse (1:700) and ATTO 488 Donkey Anti-Rabbit (1:700) for 1 hour at RT (composite green). Counterstain: Hoechst (blue) nuclear stain at 1:3000 for 1 hour at RT. Localization: Lewy body inclusions. Magnification: 20x.



Primary rat hippocampal neurons show lewy body inclusion formation when treated with active Alpha Synuclein Protein Preformed Fibrils (SPR-322) at 4 µg/ml (D-F), but not when treated with control Alpha Synuclein Protein Preformed Fibrils (SPR-317) at 4 µg/ml (A-C). Tissue: Primary hippocampal neurons. Species: Sprague-Dawley rat. Fixation: 4% formaldehyde from PFA. Primary Antibody: Mouse anti-pSer129 Antibody at 1:1000 24 hours at 4°C. Secondary Antibody: FITC Goat Anti-Mouse (green) at 1:700 for 1 hours at RT. Counterstain: Hoechst (blue) nuclear stain at 1:4000 for 1 hour at RT. Localization: Lewy body inclusions. Magnification: 20x.