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Protocols Suite — SH-SY5Y / iCell® Neurons / High-Content Imaging

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We use this protocol and it's working

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Abstract

Routine maintenance of SH-SY5Y cells and SH-SY5Y lines overexpressing 1K/2K/3K-SNCA in DMEM/F-12 with GlutaMAX, 10% FBS, and 1× Pen/Strep.

Differentiation of SH-SY5Y (WT or 3K-SNCA) using retinoic acid (RA) plus BDNF to generate neuron-like cells for downstream assays.

Culture of iCell® DopaNeurons with heterozygous SNCA p.A53T (Fujifilm CDI R1109) and isogenic control (R1088) using vendor media; optional 7-day compound treatment with half-medium changes.

Short-term (24 h) and extended (7-day) treatments in SH-SY5Y and iCell® neurons.

Fixation, permeabilisation, and immunostaining for α-syn, pSer129 α-syn, TFEB, GBA, LAMP1; DAPI nuclear stain; acquisition on Opera Phenix® Plus; analysis in Harmony/Columbus.

Materials

- SH-SY5Y (ATCC CRL-2266; RRID:CVCL_0019)
- DMEM/F-12, GlutaMAX™ (Gibco 31331-028)
- FBS, qualified (Gibco 10270-106)
- Penicillin–Streptomycin (10,000 U/mL) (Gibco 15140-122)
- Trypsin 2.5% (Gibco 15090-046)
- Versene® (EDTA) 1× (Gibco 15040-066)
- PBS, sterile (1×)
- Tissue culture plastics (T-25/T-75 flasks; 6-/12-/96-well plates)
- CO_2 incubator (37 °C, 5% CO_2), Class II hood, centrifuge, inverted microscope
- Neurobasal™ Medium (Gibco 21103-049)
- B-27™ Supplement (50×) (Gibco 17504-044)
- GlutaMAX™ Supplement (Gibco 35050-061)
- Retinoic acid (RA) (prepare 30 µM working)
- Recombinant human BDNF (Cell Signaling 3897 / 3897S) — use 5 ng/mL
- Geltrex® LDEV-free (Gibco A1413301 / A1413302)
- iCell® DopaNeurons PD SNCA A53T HZ (FCDI R1109)
- iCell® DopaNeurons (isogenic control) (FCDI R1088)
- iCell® Neural Medium (FCDI M0100)
- iCell® Neural Supplement B (FCDI M1029)
- iCell® Nervous System Supplement (FCDI M1031)
- Vendor-recommended coating (per current datasheet)
- YM201636 (Cayman Chemical 13576) — make 10 mM stock in DMSO
- Ambroxol hydrochloride (Merck/Sigma A9797) — make 10 mM stock in DMSO
- Sterile DMSO (vehicle), sterile PBS
- SH-SY5Y or differentiated SH-SY5Y; iCell® neurons
- 4% Paraformaldehyde (PFA) in PBS (Severn Biotech 40-7401-05)
- Methanol, ice-cold (100%)
- Normal Goat Serum (NGS) 5% (Abcam ab7481)
- α-Synuclein, rabbit mAb (Abcam ab138501; RRID:AB_2537217) (1:750)
- GBA, mouse mAb clone 2E2 (Millipore AP1140-100UG; RRID:AB_11211799) (1:1000)
- TFEB, rabbit mAb D2O7D (Cell Signaling #37785) (1:500)
- LAMP1, mouse mAb clone 25 (BD 611042; RRID:AB_398355) (1:1000)
- α-Synuclein, mouse mAb clone 4D6 (Abcam ab1903; RRID:AB_302665) (1:750)
- Phospho-Ser129 α-syn, mouse mAb (FUJIFILM Wako 015-25191; RRID:AB_2572224) (1:1000)
- Goat anti-mouse Alexa Fluor 488 (A-11017; RRID:AB_2534084) (1:2000)
- Goat anti-rabbit Alexa Fluor 568 (A-11011; RRID:AB_143157) (1:2000)
- Goat anti-rabbit Alexa Fluor 647 (A-21244; RRID:AB_2535812) (1:2000)
- DAPI (Thermo D1306) — 1 µg/mL, 10 min
- Opera Phenix® Plus High-Content Screening System (RRID:SCR_021100)
- Harmony® High-Content Imaging & Analysis Software (RRID per site)
- Columbus® Image Data Storage & Analysis (web-based)

Safety warnings

Follow BSL-2 aseptic technique; wear appropriate PPE.
RA is light-sensitive and teratogenic; handle under low light and use PPE.
DMSO facilitates skin absorption; YM201636 perturbs endolysosomal trafficking. Use PPE.
PFA and methanol are hazardous; use a fume hood and appropriate PPE.

Before start

- Thaw FBS and supplements at 4 °C; warm media to room temperature.
- Coating: Dilute Geltrex 1:100 in cold DMEM/F-12 on ice; add to plates and incubate 1 h RT (or overnight 4 °C). Aspirate immediately before seeding.

- Review the latest FCDI handling guide for substrate and thawing specifics.

Procedure — Staining

1 Remove medium; rinse once with PBS.
Fix with 100 µL/well 4% PFA, 20 min RT.
Permeabilise with 100 µL ice-cold methanol, 20 min.
Wash with PBS 3×.
Block with 5% NGS in PBS, 1 h RT.
Primary antibody in blocking buffer; incubate overnight at 4 °C.
Wash PBS 3×.
Secondary antibody (1:2000 in block), 2 h RT, protect from light.
Wash PBS 3×.
DAPI 1 µg/mL, 10 min → wash PBS 3× → leave 200 µL PBS/well. Store 4 °C, dark, until imaging.

Procedure — High-Content Imaging (Opera Phenix® Plus)

2 Objective: 63×; Mode: Confocal.
Fields: Acquire 5–10 random fields/well.
Channels (separate acquisitions): DAPI ~50 ms/~30% laser; AF488 ~100 ms/~70%; AF568 ~100 ms/~70%; AF647 ~100 ms/~80%.
Z-stack: 2 µm step; compute Maximum-Intensity Projection (MIP).
Save raw images with plate, well, field, channel, exposure metadata.

Procedure — Image Analysis (Columbus/Harmony)

3 Import ▯ MIP z-stacks (if not already done in acquisition).
Segment nuclei on DAPI (Columbus Method B).
Define cytoplasm expanding from nuclei; use α-syn (488) to assist (Columbus Method A).
α-Syn inclusions (488): Cytoplasmic mask; intensity filter 5,500–20,000 a.u.; extract # inclusions/field and MFI of foci; normalise by nuclei count → inclusions/cell.
TFEB partitioning (568): Nuclear vs cytoplasmic ROIs; intensity range 150–20,000 a.u.; compute MFI/cell for nuclei and cytoplasm (normalise by nuclei).
GBA (568): Cytoplasmic intensity range 600–20,000 a.u.; mean cytoplasmic intensity/cell.
LAMP1 (568): Cytoplasmic mask; 600–20,000 a.u.; raw sum intensity/cell (or puncta counts if spot detection enabled).
pSer129 α-syn (488): Cytoplasmic mask; 1,000–1,800 a.u.; # inclusions/cell.
QC ▯ Export: Exclude fields with ▯50 cells or saturated pixels; export per-field, per-well summaries for statistics.

Expected results

4 Robust nuclear DAPI; discrete α-syn cytoplasmic inclusions when present; measurable TFEB nuclear/cytoplasmic shift; LAMP1 puncta in perinuclear region.

Troubleshooting

5 Weak signal — verify antibody dilutions and fixation/permeabilisation consistency; adjust exposure to avoid saturation.