PARK-PINK U2OS CELL LINE

PARK2-FP602+tGFP-Pink1 U2OS

Experimental Assay

1. Introduction

Park2-FP602 + tGFP-Pink1 U2OS cell line was designed to study mitophagy and Parkinson's disease mechanisms enabling the tracking of their recruitment to mitochondria upon depolarization. The fluorescence tags facilitates high-throughput drug screening to identify neuroprotective compounds that modulate Park2 and Pink1 activity providing valuable insights into neurodegenerative disease processes.

2. Product components and recommended storage conditions

- U2OS Parkin-FP602 + tGFP-Pink1 (Innoprot P30711)
- 1 vial $3x10^6$ cells in Freezing Media
- Storage: Immediately upon receipt, storage in liquid nitrogen

3. Biological Activity

- This cell line has been validated for cellular response to stimulation with FCCP (Tocris 453).
- Mycoplasma testing: The cell line has been screened using a Mycoplasma PCR Detection Kit (Abm G238) following manufacturer´s instructions.

4. Recommended Reagents to Be Supplied by the User

- DMEM/Nutrient Mixture F-12 Ham (Sigma-Aldrich D8437)
- Fetal bovine serum (FBS) (Sigma-Aldrich F7524)
- DPBS with calcium and magnesium (Sigma Aldrich D8662)
- Opti-MEM - Glutamax (ThermoScientific 51985-026)
- Greiner CELLSTAR® 96 well plates flat bottom black polystyrene wells (with micro-clear bottom) (Greiner M0562-32EA)
- Hoechst 33342 (ThermoFisher H1399)

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5. Recommended Equipments

- Class II biological safety cabinet
- Hemacytometer / Cell counter
- Incubator humidified 37°C, 5% CO_2
- Inverted microscope
- Image analysis: Appropriate filter for the turboFP602 protein fluorescent signal, with excitation an emission peaks at 574 nm and 602 nm, respectively and tGFP protein with excitation an emission peaks at 482 and 502 nM, respectively.

6. Image Assay: Experimental Protocol

- Day 1: Thaw the cell line (3×10^6 cells per T25 flask).
- Day 2: Maintain cells in DMEM-F12 with 10% FBS at 37 °C in a humidified 5% CO_2 incubator.
- Day 3: Plate 20,000 cells per well in a 96-well plate and incubate in DMEM-F12 + 10% FBS for 24 hours at 37 °C, 5% CO_2.
- Day 4: Treat cells with FCCP diluted in OptiMEM and incubate for 90 minutes. Add Hoechst at a final concentration of 10-20 µg/ml directly to the wells without removing the media and incubate for 20–30 minutes at 37 °C, 5% CO_2.
- Analysis: Replace the medium with 100 µL PBS and proceed with fluorescence imaging. Parkin recruitment is assessed by counting the spot number and Pink relocalization by measuring mitochondrial average area.

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