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Flow cytometry-based measurement of mitophagic flux

In 1 collection

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ABSTRACT

Protocol for flow cytometry-based measurement of mitophagic flux

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Felix Kraus 2022. Flow cytometry-based measurement of mitophagic flux. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ge34jyl47/v1

COLLECTIONS

Kraus et al., 2022 FBXO7 /Park15

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Kraus et al., 2022 FBXO7 /Park15

Generation of stable mtKeima cell lines

1 Generate stable cell lines expressing mitochondrial targeted mKeima. See dx.doi.org/10.17504/protocols.io.br87m9zn and dx.doi.org/10.17504/protocols.io.6qpvr4xn3gmk/v1

Seeding of HeLa cells

2 Wash HeLa cells expressing doxycycline-inducible Parkin with 1x PBS

3 Add Trypsin to cells for 5 min and incubate at 37°C to dissociate cells from plastic well

4 Resuspend cells in 1 mL DMEM media

5 Count cells

6 Seed appropriate number of cells into 24-well glass bottom dish

7 Top up glass bottom dish with either 1 mL DMEM and place cells back into incubator

8 The next day exchange DMEM with DMEM + 2µg/ml doxycycline for 18h to induce Parkin expression.

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9 Induce mitophagy using Antimycin A / Oligomycin A for the desired time. Also have BafA (25nM) sample for normalization.
==> proceed to step 17 for flow cytometry-based measurements.

Differentiation of iNeurons

10 Day 0: Treat AAVS1-TRE3G-NGN2 cells with Accutase and plate the dissociated cells in matrigel-coated 6-well plates (2x105 cells/well) in ND1 Medium supplemented with Y27632 (10 µM). ND1 Medium: DMEM/F12 N2 (100x) 1x BDNF 10 ng/ml NT3 10 ng/ml NEAA (100X) 1x Laminin 0.2 µg/ml Doxycycline 2 µg/ml

11 Day 1: Replace the medium with ND1 Medium.

12 Day 2: Replace the medium with ND2 Medium. ND2 Medium Neurobasal medium B27 (50x) 1x GlutaMax (100x) 1x BDNF 10 ng/ml NT3 10 ng/ml Doxycycline 2 µg/ml

13 Day 4: Exchange 50% of the medium from each well.

14 Day 6: Treat the cells with Accutase and replate the dissociated cells in matrigel-coated 6-/12-well glass bottom plates (2-4x105 cells/well for 6 wells) in ND2 Medium.

15 Day 8 and thereafter: Exchange 50% of the medium from each well every other day. · Doxycycline can be withdrawn on Day.

16 Induce mitophagy using Antimycin A / Oligomycin A for the desired time. Also have BafA (25nM) sample for normalization.

Keima mito flux analysis

17 Detach cells from vessel of choice and resuspend carefully so they do not clump. If required, filtered through a cell strainer cap tube. Place in vessel of choice for cytometry, for example tubes or 96 well plates.

18 Use dual-excitation (440nm for ph7 and 561nm for pH3) and collect in 620 nm range. Analyze at least 10.000 single, healthy cells. Calculate of acidic:neutral mt-Keima ratio on a per-cell

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basis in FlowJo Software.
If have BafA sample, use as normalization for sample set in genotype, otherwise use fed control.

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