Oct 31, 2022

Microscopy-based evaluation of Parkin translocation and mitophagy in FBXO7-/- cell linesons)

In 1 collection

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dx.doi.org/10.17504/protocols.io.yxmvm2kqog3p/v1

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ABSTRACT

Protocol for the microscopy-based evaluation of Parkin translocation and mitophagy in FBXO7-/- cell linesons)

DOI

dx.doi.org/10.17504/protocols.io.yxmvm2kqog3p/v1

PROTOCOL CITATION

Felix Kraus 2022. Microscopy-based evaluation of Parkin translocation and mitophagy in FBXO7-/- cell linesons). **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm2kqog3p/v1

COLLECTIONS

Kraus et al., 2022 FBXO7 /Park15

CREATED

Oct 11, 2022

LAST MODIFIED

Oct 31, 2022

PROTOCOL INTEGER ID

71184

PARENT PROTOCOLS

Part of collection

Kraus et al., 2022 FBXO7 /Park15

23 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

23 Day 1: Replace the medium with ND1 Medium.

23 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

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

23 Day 6: Treat the cells with Accutase and replate the dissociated cells in matrigel-coated

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23 6-/12-well glass bottom plates (2-4x105 cells/well for 6 wells) in ND2 Medium.

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 · Doxycycline can be withdrawn on Day.

23 Mount glass bottom plate on Yokogawa CSU-W1 spinning disk confocal on a Nikon Eclipse Ti-E motorized microscope. Ensure that the system is equipped with a Tokai Hit stage top incubator and imaging was performed at 37°C, 5% CO2 and 95% humidity.

23 Take image stacks using a Nikon Plan Apo 60×/1.40 N.A immersion oil objective lens.

23 For ratiometic imaging, mtKeimaXL were excited in sequential manner with a Nikon LUN-F XL solid state laser combiner ([laser line – laser power]: 445 - 80mW, 561 – 65 mW]) using a Semrock Di01-T445/515/561 dichroic mirror. Fluorescence emissions were collected through a Chroma ET605/52m [for 445 nm] and a 568 Chroma ET605/52m [for 561 nm], filters, respectively (Chroma Technologies). Consistent laser intensity and exposure times must be maintained for all samples.

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23 Perform image quantification was in your tool of choice. Here we will use ImageJ/FiJi and custom-written batch-macros (https://github.com/harperlaboratory/FBXO7)

23 Divide raw confocal images of mitochondrial targeted mt-mKeimaXL [ex:561/ex:445], resulting in a ratiometic image of only acidic Keima-puncta.

23 Subject resulting signals to background subtraction (rolling kernel size 25, sliding paraboloid) and convert into binary objects.

23 Use the "Analyze Particles..." command (pixel size exclusion: 0.5-∞, exclude edge objects) to measure foci-abundance and other morphological parameters.

23 Save results for each image-stack as .csv files, together with the original ratiometic .tiff file for QC purposes.

23 Count number of nuclei for normalization.

23 Plot results in your tool of choice for graphing and statistical analysis.

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