

Jan 24, 2023

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

DOI

<https://dx.doi.org/10.17504/protocols.io.dm6gpjw28gzp/v1>

Felix Kraus¹

¹Harvard Medical School



Felix Kraus

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Protocol Citation: Felix Kraus 2023. Microscopy-based evaluation of Parkin translocation and mitophagy in FBXO7-/- cell lines. protocols.io <https://dx.doi.org/10.17504/protocols.io.dm6gpjw28gzp/v1>

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Protocol status: In development

We are still developing and optimizing this protocol



Created: October 11, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 71185

Keywords: ASAPCRN, evaluation of parkin translocation, parkin translocation, mitophagy, microscopy

Funders Acknowledgements:

ASAP

Grant ID: ASAP-000282

Abstract

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



Seeding of HeLa

- 1 Wash HeLa cells expressing GFP-Parkin with 1x PBS
- 2 Add Trypsin to cells for 5 min and incubate at 37°C to dissociate cells from plastic well
- 3 Resuspend cells in 1 mL DMEM media
- 4 Count cells
- 5 Seed appropriate number of cells into 24-well glass bottom dish
- 6 Top up glass bottom dish with either 1 mL DMEM and place cells back into incubator
- 7 Induce mitophagy using Antimycin A / Oligomycin A for the desired time.

Staining

- 8 Aspirate DMEM and fix cells in 1 ml pre-warmed 4% PFA for 30 min.
- 9 Aspirate PFA solution and wash wells 3x with PBST (1x PBS, 0.02% Tween 20)
- 10 Permeabilize the cells by adding 0.2% Triton X-100 in PBS.
- 11 Remove the detergent solution by aspiration. Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.
- 12 Block cells for 10 min with 3% BSA – 1x PBS.



- 13 Remove BSA solution by aspiration. Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.
- 14 Incubate with primary antibodies in 3% BSA - 1x PBS for 3h at RT with gentle shaking. a. Anti-GFP (chicken) b. Anti-HSP60 (mouse)
- 15 Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.
- 16 Incubate with secondary antibodies in 3% BSA - 1x PBS for 45 min – 1h. a. Goat anti-chicken AlexaFlour 488 b. Goat anti-mouse AlexaFluor 647 c. DNA-SPY555
- 17 Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.
- 18 Wash wells 3x with PBST (1x PBS, 0.02% Tween 20). Drain well.
- 19 Exchange PBST with 1x PBS and keep cells at 4°C until imaging. Image within the next few days.

Fixed-cell microscopy

- 20 Mount glass bottom plate on Yokogawa CSU-W1 spinning disk confocal on a Nikon Eclipse Ti-E motorized microscope equipped with a Nikon Apochromat 60×/1.42 N.A oil-objective lens. Image signals of 488/568/647 fluorophores in sequential manner with a Nikon LUN-F XL solid state laser combiner ([laser line – laser power]: 488 - 80mW, 561 - 65mW, 640nm - 60mW) using a Semrock Di01-T405/488/568/647 dichroic mirror. Fluorescence emissions were collected with 488 Chroma ET525/50m [488 nm], 568 Chroma ET605/52m [561 nm], 633 Chroma ET705/72m [640 nm] filters, respectively (Chroma Technologies) using NIS-Elements image acquisition software. Consistent laser intensity and exposure times must be maintained for all samples. Acquire 8 µm z-stacks for each image.
- 21 Image adequate number of cells per repeat in each condition.

Evaluation



- 22 Perform image quantification was in your tool of choice. Here we will use CellProfiler and segmentation pipelines can be found online (<https://github.com/harperlaboratory/FBXO7>).
- 23 Export results and plot in your tool of choice for graphing and statistical analysis.