JAN 23, 2023

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

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ABSTRACT

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

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.dm6gpjw28gzp/v1

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

Protocol status: In development
We are still developing and optimizing this protocol

Created: Oct 11, 2022

Last Modified: Jan 23, 2023

PROTOCOL integer ID: 71185

Keywords: ASAPCRN

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.