




Oct 31, 2022

🌐 Microscopy-based pUb-coverage measurements of mitochondria in iNeurons

 In 1 collection**Felix Kraus**^{1,2}¹Department of Cell Biology, Blavatnik Institute, Harvard Medical School, 240 Longwood Ave, Boston MA 02115, USA;²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA1 *Works for me* Sharedx.doi.org/10.17504/protocols.io.e6nvwj529lmk/v1 Felix Kraus

ABSTRACT

Protocol for microscopy-based pUb-coverage measurements of mitochondria in iNeurons

DOI

dx.doi.org/10.17504/protocols.io.e6nvwj529lmk/v1

PROTOCOL CITATION

Felix Kraus 2022. Microscopy-based pUb-coverage measurements of mitochondria in iNeurons. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.e6nvwj529lmk/v1>



COLLECTIONS ⓘ

 **Kraus et al., 2022 FBX07 /Park15**

LICENSE

_____ This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Oct 11, 2022

LAST MODIFIED

Oct 31, 2022

- 22 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

- 22 Day 1: Replace the medium with ND1 Medium.

- 22 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

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

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

- 22 6-/12-well glass bottom plates (2-4x10⁵ cells/well for 6 wells) in ND2 Medium.

- 22 Day 8 and thereafter: Exchange 50% of the medium from each well every other day. Doxycycline can be withdrawn on Day.
- 22 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.
- 22 Take image stacks using a Nikon Plan Apo 60×/1.40 N.A immersion oil objective lens.
- 22 For ratiometric 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.
- 22 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.
- 22 Take image stacks using a Nikon Plan Apo 60×/1.40 N.A immersion oil objective lens.
- 22 For ratiometric 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.
- 22 Perform image quantification was in your tool of choice. Here we will use ImageJ/Fiji and custom-written batch-macros (<https://github.com/harperlaboratory/FBX07>)
- 22 Divide raw confocal images of mitochondrial targeted mt-mKeimaXL [ex:561/ex:445], resulting in a ratiometric image of only acidic Keima-puncta.

- 22 Subject resulting signals to background subtraction (rolling kernel size 25, sliding paraboloid) and convert into binary objects.
- 22 Use the "Analyze Particles..." command (pixel size exclusion: 0.5- ∞ , exclude edge objects) to measure foci-abundance and other morphological parameters.
- 22 Save results for each image-stack as .csv files, together with the original ratiometric .tiff file for QC purposes.
- 22 Count number of nuclei for normalization.
- 22 Plot results in your tool of choice for graphing and statistical analysis.