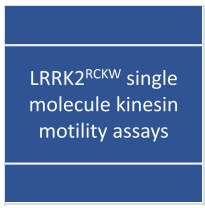

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LRRK2^{RCKW} single molecule kinesin motility assays

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This protocol is about LRRK2^{RCKW} single molecule kinesin motility assays.

LRRK2RCKW singlemolecule kinesin assay.pdf

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LRRK2, motility assay, kinesin, imaging, single-molecule, ASAPCRN

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As used in:

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https://doi.org/10.1038/s41586-020-2673-2

Image Recommendation:
Settings will vary per microscope. We imaged K560-GFP every 500 msecs for 2 mins with 25% laser (488) power at 150 ms exposure time. Each sample was imaged no longer than 15 mins. Each technical replicate recommended to consist of movies from at least two fields of view containing between 5 and 10 microtubules each.

Image using the 405 nm laser to determine the locations of the microtubules. Preferably at the start and end of the experiment.

Single-molecule motility assay analysis recommendation:
Kymographs were generated from motility movies and quantified for run lengths, percent motility, and velocity using ImageJ (NIH). Specifically, maximum-intensity projections were generated from time-lapse sequences to define the trajectory of particles on a single microtubule. The segmented line tool was used to trace the trajectories and map them onto the original video sequence, which was subsequently re-sliced to generate a kymograph. Motile and immotile events (> 1 sec) were manually traced. Bright aggregates, which were less than 5% of the population, were excluded from the analysis. Run length measurements were calculated from motile events only. For percent motility per microtubule measurements, motile events (> 1 sec and > 1μm) were divided by total events per kymograph. Velocity measurements were calculated from the inverse slopes of the motile event traces (> 1 sec and > 1μm) only. Statistical analyses were performed in Prism8 (Graphpad).

Recommended Equipment and Setup:
This single-molecule imaging experiment was originally performed using total internal reflection fluorescence (TIRF) microscopy with an inverted microscope (Nikon, Ti-E Eclipse) equipped with a 100x 1.49 N.A. oil immersion objective (Nikon, Plano Apo), and a MLC400B laser launch (Agilent), with 405 nm, 488 nm, 561 nm and 640 nm laser lines (561 and 640 nm laser lines are not needed for this version of the experiment). Excitation and emission paths were filtered using single bandpass filter cubes (Chroma), and emitted signals were detected with an electron multiplying CCD camera (Andor Technology, iXon Ultra 888). Illumination and image acquisition were controlled with NIS Elements Advanced Research software (Nikon), and the xy position of the stage was controlled with a ProScan linear motor stage controller (Prior).

Required Buffers:

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Streptavidin Buffer:

- [M]1 mg/mL Streptavidin
- [M]30 millimolar (mM) HEPES pH 7.4
- [M]2 millimolar (mM) MgOAc
- [M]1 millimolar (mM) EGTA
- [M]10 % Glycerol

Motility Assay Buffer:

- [M]30 millimolar (mM) HEPES pH 7.4
- [M]50 millimolar (mM) KOAc
- [M]2 millimolar (mM) MgOAc
- [M]1 millimolar (mM) EGTA
- [M]10 % Glycerol
- [M]1 millimolar (mM) DTT
- [M]20 micromolar (µM) Taxol

Motility Assay Buffer with casein:

- [M]30 millimolar (mM) HEPES pH 7.4
- [M]50 millimolar (mM) KOAc
- [M]2 millimolar (mM) MgOAc
- [M]1 millimolar (mM) EGTA
- [M]10 % Glycerol
- [M]1 millimolar (mM) DTT
- [M]20 micromolar (µM) Taxol
- [M]1 mg/mL casein

LRRK2 Buffer:

- [M]20 millimolar (mM) HEPES pH 7.4
- [M]80 millimolar (mM) NaCl
- [M]0.5 millimolar (mM) TCEP
- [M]5 % Glycerol
- [M]2.5 millimolar (mM) MgCl2
- [M]20 micromolar (µM) GDP

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For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Please take notice of the buffer preparation in section 'Materials'.
Make sure that you have labeled taxol-stabilized microtubules available. See the protocol here.

Create microscope slides: 1h 11m

1 Adhere Biotin-PEG-functionalized coverslips (Microsurfaces) to a microscope slide using double-sided scotch tape, creating 4 channels per slide.

2 Add the streptavidin buffer to each channel and incubate for **00:03:00** . 3m

3 

Wash twice with Motility Assay buffer.

4  3m

Add a 1:150 dilution of taxol-stabilized microtubules (**19 µL** per channel) and incubate for **00:03:00** .
See https://dx.doi.org/10.17504/protocols.io.bp2l6bdedgqe/v1 for making taxol-stabilized microtubules.

5 

Wash twice with LRRK2 buffer. Add more buffer if necessary to prevent drying out.

Prepare LRRK2: 1h 11m

6 

Prepare a **[M]1 micromolar (µM)** solution of LRRK2^{RCKW} in a cold LRRK2 buffer. Centrifuge through a 0.1 µm PVDF filter to remove aggregates. Calculate the new effective concentration. Usually around **[M]500 nanomolar (nM)** - **[M]700 nanomolar (nM)** after centrifugation.

7  10m

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Create a working aliquot of LRRK2 in the desired concentration (ex. [M]**25 nanomolar (nM)** - [M]**100 nanomolar (nM)**) in the LRRK2 buffer at **Room temperature** (recommended volume of **25 µL**). If adding inhibitors, add them now with DMSO. Incubate for **00:10:00** at **Room temperature** .

Next steps: 5m

5m

8 

Add LRRK2^{RCKW} sample to the channel (**19 µL**). Incubate for **00:05:00** . Prepare next step while waiting.

9 

Wash twice with the motility assay buffer supplemented with [M]**1 mg/mL casein** .

Prepare kinesin:

10 

Make a [M]**4 nanomolar (nM)** solution of K560-GFP in the Motility Assay buffer with casein supplemented with an oxygen scavenger system ([M]**0.4 % glucose** , [M]**45 µg/mL glucose catalase** (Sigma-Aldrich), and [M]**1.15 mg/mL glucose oxidase** (Sigma-Aldrich)), [M]**71.5 millimolar (mM) beta-mercaptoethanol** and [M]**1 millimolar (mM) ATP** .

Next steps:

11 Add **19 µL kinesin mixture** to each chamber.

12 Image slide.

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