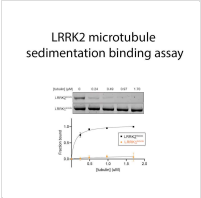

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LRRK2 microtubule sedimentation binding assay

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Mariusz Matyszewski

Assay to determine LRRK2 protein binding to microtubules.

Original assay by Andrea Dickey. Adapted by Mariusz Matyszewski for protocols.io.

Assay originally used in Snead, Matyszewski, Dickey et al. 2022

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LRRK2, microtubule, binding

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Materials:

- Porcine brain tubulin purchased from Cytoskeleton, Inc.
- Purified LRRK2^{RCKW}

Buffers:

LRRK2 Binding Buffer:

- [M]**20 millimolar (mM) HEPES pH 7.4**
- [M]**220 millimolar (mM) NaCl**
- [M]**0.5 millimolar (mM) TCEP**
- [M]**5 % volume glycerol**
- [M]**2.5 millimolar (mM) MgCl2**
- [M]**20 micromolar (µM) GDP**
- [M]**20 micromolar (µM) Taxol**

Microtubule preparation 25m

1 Polymerize tubulin at around [M]**2.5 mg/mL** for **00:30:00** at **37 °C** . Add Taxol for stabilization and incubate for another **00:10:00** at **37 °C** . 40m

2 Remove free tubulin by ultracentrifugation. **108628 x g, 37°C, 00:15:00** through a [M]**64 % volume glycerol cushion** . 15m

3 Resuspend the resulting microtubule pellet in the **LRRK2 binding buffer**.

3.1 Determine microtubule concentration by running an SDS-PAGE with actin standards.

4 Incubate desired amount of LRRK2^{RCKW} protein ([M]**200 nanomolar (nM)** in our experiments) at **Room temperature** for **00:10:00** with varied concentrations of microtubules in the **LRRK2 binding buffer**. 10m

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Assay can be modified to work with other proteins. We used the same assay to monitor LRRK1^{RCKW} binding.

5 Pellet the microtubules by ultracentrifugation. **108628 x g, 25°C, 00:15:00** 15m

6 Quantify the depletion of LRRK2^{RCKW} by taking the supernatant and boiling for **00:10:00** in SDS containing buffer for running a gel. 10m

7 Samples were run on 4-12% polyacrylamide gels (NuPage, Invitrogen) and stained with SYPRO-Red Protein Gel Stain (ThermoFisher) for protein detection.
Binding curves were fit in GraphPad Prism (9.2; GraphPad Software) with a nonlinear regression hyperbolic curve.

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