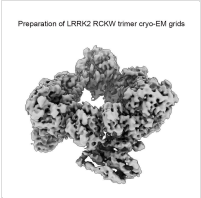

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Preparation of LRRK2 RCKW trimer cryo-EM grids

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ABSTRACT

This protocol has been adapted from Deniston et al (https://doi.org/10.1038/s41586-020-2673-2)

Original protocol by Colin Deniston. Adapted to protocol.io by Mariusz Matyszewski.

This protocol describes how to create cryo-EM grids for LRRK2 RCKW. In particular, this protocol was used to obtain a high-resolution cryo-EM structure of the LRRK2 RCKW trimer, as well as lower resolution structures of RCKW monomers and dimers.

PROTOCOL CITATION

Mariusz Matyszewski 2022. Preparation of LRRK2 RCKW trimer cryo-EM grids. **protocols.io** https://protocols.io/view/preparation-of-lrrk2-rckw-trimer-cryo-em-grids-brypm7vn

KEYWORDS

cryo-EM, LRRK2, structural biology, ASAPCRN

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GUIDELINES

Newer protocol is available (see updated protocol here) with better results for monomeric protein. This is included mainly for archival reasons.

MATERIALS TEXT

LRRK2 Buffer:

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

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).
Take proper precautions while freezing grids.

Preparing Sample

1 Dialyze purified **LRRK2 RCKW** into the final LRRK2 buffer (see Materials)

2 Dilute the protein to the desired final concentration.

High concentrations favor dimers and trimers, while lower concentrations favor monomers. For the published trimer structure, a final concentration of 4 uM was used (see Deniston et al).

2.1 **(Optional)** If adding inhibitors, add them after diluting to the final concentration and incubate on ice for **01:00:00** . If using Apo protein, then proceed immediately. 1h

Freezing Grids 20s

3 Glow discharge grids. 20s
We used UltrAuFoil Holey Gold 1.2/1.3 300 mesh grids and glow discharged them at 20 mA for **00:00:20** in a K100 instrument.

4 Apply protein to grids and plunge freeze.
We used a Vitrobot (FEI) to blot away excess sample and plunge freeze

5 Store grids in liquid nitrogen until ready for imaging.