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## LRRK2:DARPin complex preparation

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**We use this protocol and it's working**

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## Abstract

Protocol used to create LRRK2-RCKW: DARPin complex for cryo-EM grid preparation.

## LRRK2:DARPin complex preparation

1

His6-Z-TEV-LRRK2-RCKW was expressed and purified as described in a previous protocol

### Protocol



NAME

LRRK2 RCKW Protein Purification

CREATED BY

Mariusz Matyszewski

Preview

2

**Prepare LRRK2 buffer exchange: Keep it at 4°C.**

20 millimolar (mM) HEPES pH=7.4

150 millimolar (mM) NaCl

2.5 millimolar (mM) MgCl<sub>2</sub>

20 micromolar (μM) GDP

0.5 millimolar (mM) TCEP

3

Spin down purified LRRK2-RCKW (10000 rcf, 4°C, 10 minutes). Leave protein on ice afterward.

**Note**

For the best result, keep protein on ice and reduce the amount of time between spinning and freezing cryo-EM samples.

- 4 Exchange buffer using a spin desalting column (Zeba™ Spin Desalting Columns, 7K MWCO (Catalog number: 89877)).
- 5 Spin down again the exchange buffer LRRK2-RCKW (10000 rcf, 4°C, 10 minutes) and measure the concentration. Leave protein on ice afterward
- 6 Thaw the DARPin protein of your interest (E11 or C12) and spin it down. Measure its concentration.
- 7 Based on LRRK2-RCKW concentration, add the necessary volume to get a proportional ratio  
LRRK2:DARPin 1:1.25 and dilute to a final 10 micromolar ( $\mu\text{M}$ ) LRRK2-RCKW concentration using exchange buffer (150 mM NaCl)
- 8 Incubate 10 minutes at RT. Afterward, keep it on ice until grid preparation.