

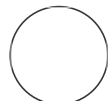


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LRRK2-RCKW: MLI-2: E11 DARPin cryo-EM sample preparation

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ABSTRACT

Protocol used to prepare LRRK2-RCKW: DARPin:MLi-2 complex and cryo-EM grid preparation.

BEFORE START INSTRUCTIONS

Make

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We use this protocol and it's working

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Protein purification and buffer exchange

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

Protocol



NAME

LRRK1 expression and purification

CREATED BY

Robert Fagiewicz

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) MgCl₂
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. 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

Expected result

The initial concentration range was 20-40 μM . The final concentration might be half of the initial one. Final volume might be 13-16 μL .

- 5.1 Thaw E11 DARPin and spin it down. Measure its concentration.

- 6 Dilute MLi-2 stock (diluted in 100% DMSO) to a desired concentration

- 7 Based on LRRK2-RCKW concentration, add the necessary volume to get a proportional ratio LRRK2:DARPin:MLi-2 1:1.25:3 and dilute to a final 10 micromolar (μM) LRRK2-RCKW concentration using exchange buffer (150 mM NaCl).

- 8 Incubate 10 minutes at RT. Afterward, keep it on ice until grid preparation.

E11 DARPin purification

- 9 E11 DARPin purified as described in the next protocol

cryo-EM sample preparation

- 10 We used UltraAuFoil Holey Gold 2/2 200 mesh grids and plasma cleaning them in the Solarus II (Gatan) using the QuantiFoil Au preset.

- 11 Dilute the sample to the desired concentration using the LRRK2 exchange buffer. We used 6 micromolar (μM).
- 12 Apply 3 to 3.5 microliters (μl) of sample and plunge freeze. We used a Vitrobot (FEI) to blot away excess sample and plunge freeze in ethane liquid. (In our case, we use 4 seconds as a time blot as 20 sec as a wait time and 4 as a blot force, but these parameters are slightly different from one Vitrobot to another. I would try with the Vitrobot parameters already tested in your machine first).
- 13 Store grids in liquid nitrogen until ready for imaging