May 29, 2024

LRRK2:DARPins complex preparation

DOI
dx.doi.org/10.17504/protocols.io.j8nlkowxxv5r/v1

Marta Sanz Murillo[1,2]

[1]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093, USA;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA

ASAP Collaborative Rese…

Marta Sanz Murillo
University of California, San Diego

DOI: dx.doi.org/10.17504/protocols.io.j8nlkowxxv5r/v1

Protocol Citation: Marta Sanz Murillo 2024. LRRK2:DARPins complex preparation. **protocols.io https://dx.doi.org/10.17504/protocols.io.j8nlkowxxv5r/v1**

Protocol status: Working
We use this protocol and it's working

Created: August 10, 2023

Last Modified: May 29, 2024

Protocol Integer ID: 86349

Keywords: ASAPCRN, cryo-EM, LRRK2, structural biology

Funders Acknowledgement:
Aligning Science Across Parkinson's: ASAP
Grant ID: ASAP-000519

Abstract

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

LRRK2:DARPins 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 4ºC.**

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.

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 (µM) LRRK2-RCKW concentration using exchange buffer (150 mM NaCl)

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