

Sep 24, 2022

Assay for Dual Rab GTPase binding to the LRRK2 Armadillo Domain

DOI

<https://dx.doi.org/10.17504/protocols.io.81wgbypzovpk/v1>

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Protocol Citation: Claire Chiang, Suzanne Pfeffer 2022. Assay for Dual Rab GTPase binding to the LRRK2 Armadillo Domain.
protocols.io <https://dx.doi.org/10.17504/protocols.io.81wgbypzovpk/v1>

Manuscript citation:

<https://doi.org/10.7554/eLife.79771>

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Protocol status: Working

We use this protocol and it's working

Created: September 24, 2022

Last Modified: May 31, 2024

Protocol Integer ID: 70457

Keywords: ASAPCRN, Irrk2 armadillo domain the Irrk2 armadillo domain, Irrk2 armadillo domain, multiple rab gtpase, dual rab gtpase, bind armadillo domain, armadillo domain, immobilized armadillo domain, rab8a, test if phosphorab10, binding site, phosphorab10, assay, Irrk2

Funders Acknowledgements:

Aligning Science Across Parkinson's

Grant ID: 000463

Abstract

The LRRK2 Armadillo domain contains multiple Rab GTPase binding sites. To show that the sites can be occupied simultaneously, we use this assay. The idea is to immobilize Rab8A, bind Armadillo domain, and test if phosphoRab10 can bind to Rab8A-immobilized Armadillo domain.

Materials

His-MST3 protein (pET15b 6HIS MST3 TV1; MRC-PPU DU62980)

His-Rab8A Q67L full length

His-GFP-Rab10 Q68L 1-181

Reaction buffer: 50 mM HEPES  , 150 mM NaCl, 5 mM MgCl₂, 0.2 mM TCEP, 100 μM GTP, 2 mM ATP, 5% (v/v) glycerol



Dual Rab GTPase binding to LRRK2 Armadillo Domain

3h 36m 30s

- 1 Phosphorylate His-Rab10 Q68L 1-181 with His-MST3 kinase at a molar ratio of 1:3 (kinase:substrate) at 30 °C 02:00:00 in reaction buffer. See below for details. 2h

Citation

Axel Knebel, Kerryndsen, Pawel Lis, Paul Davies, Dario R Alessi (2026)
. Expression and purification of Rab8A (1-181) stoichiometrically phosphorylated at pThr72 (the LRRK2 site).

[dx.doi.org/10.17504/protocols.io.butinwke](https://doi.org/10.17504/protocols.io.butinwke)

[LINK](#)

- 2 Pellet 50 µL glutathione agarose slurry at 3000 rpm, 4°C, 00:05:00 . 5m
- 3 Add GST-Rab8A Q67L to glutathione beads to achieve a concentration of 6 micromolar (µM) in a total volume of 50 µL reaction buffer. Incubate at Room temperature for 00:30:00 on a rotator. 30m
- 4 Pellet beads by spinning at 3200 x g, Room temperature, 00:00:30 and discard supernatant. 30s
- 5 Add His-LRRK2 Armadillo domain 1-552 (10 micromolar (µM) final in 50 µL) or buffer alone and incubate at Room temperature for 00:30:00 on a rotator. 30m
- 6 Pellet beads by spinning at 3200 x g, 00:00:30 and discard supernatant. 30s
- 7 Add phosphorylated His-Rab10 Q68L 1-181 (4 micromolar (µM) in 50 µL) and incubate at Room temperature for 00:30:00 on a rotator. 30m



- 8 Wash beads 2X with  500 μL reaction buffer.
- 9 Elute protein from beads using  50 μL elution buffer (reaction buffer +  50 millimolar (mM) reduced glutathione).
- 10 Pellet beads by spinning at  3200 x g, 00:00:30 and collect supernatant.
- 11 Analyze eluate by SDS-PAGE and immunoblot for phosphoRab10; image blots with Li-COR, and quantify bands using ImageJ (see below for details).

30s

Citation

Francesca Tonelli, Dario Alessi (2026)
. Quantitative Immunoblotting Analysis of LRRK2 Signalling Pathway.

[dx.doi.org/10.17504/protocols.io.bsgrnbv6](https://doi.org/10.17504/protocols.io.bsgrnbv6)

[LINK](#)

Citations

Step 1

Axel Knebel, Kerryndsen, Pawel Lis, Paul Davies, Dario R Alessi. Expression and purification of Rab8A (1-181) stoichiometrically phosphorylated at pThr72 (the LRRK2 site)

[dx.doi.org/10.17504/protocols.io.butinwke](https://doi.org/10.17504/protocols.io.butinwke)

Step 11

Francesca Tonelli, Dario Alessi. Quantitative Immunoblotting Analysis of LRRK2 Signalling Pathway

[dx.doi.org/10.17504/protocols.io.bsgrnbv6](https://doi.org/10.17504/protocols.io.bsgrnbv6)