Sep 23, 2022

Assay for Dual Rab GTPase binding to the LRRK2 Armadillo Domain

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dx.doi.org/10.17504/protocols.io.81wgbypzovpk/v1

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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.

DOI

dx.doi.org/10.17504/protocols.io.81wgbypzovpk/v1

PROTOCOL CITATION

Claire Y Chiang, Suzanne R Pfeffer 2022. Assay for Dual Rab GTPase binding to the LRRK2 Armadillo Domain. **protocols.io** https://protocols.io/view/assay-for-dual-rab-gtpase-binding-to-the-lrrk2-arm-cg2ztyf6

FUNDERS ACKNOWLEDGEMENT

Aligning Science Across Parkinson's
Grant ID: 000463

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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

CREATED

Sep 23, 2022

LAST MODIFIED

Sep 23, 2022

PROTOCOL INTEGER ID

70457

MATERIALS TEXT

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 pH 8, 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

> Axel Knebel, Kerryn Berndsen, Pawel Lis, Paul Davies, Dario R Alessi. Expression and purification of Rab8A (1-181) stoichiometrically phosphorylated at pThr72 (the LRRK2 site). http://dx.doi.org/10.17504/protocols.io.butinwke

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 30s

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

supernatant.

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. 30s

11 Analyze eluate by SDS-PAGE and immunoblot for phosphoRab10; image blots with Li-COR, and quantify bands using ImageJ (see below for details).

Francesca Tonelli, Dario Alessi. Quantitative Immunoblotting Analysis of LRRK2 Signalling Pathway.
http://dx.doi.org/10.17504/protocols.io.bsgrnbv6

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