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In vitro coimmunoprecipitation between CHMP2B and alpha-synuclein

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Cole Sitron¹, F Ulrich Hartl¹

¹Max Planck Institute of Biochemistry



Cole Sitron

Max Planck Institute of Biochemistry

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details an in vitro coimmunoprecipitation between recombinant CHMP2B and different alpha-synuclein species - either monomers or fibrils. The goal of the experimental setup described herein is to assess which alpha-synuclein species CHMP2B preferentially binds.

Materials

- Recombinant alpha-synuclein A53T monomers and PFFs (5 mg/ml; prepared as in [dx.doi.org/10.17504/protocols.io.btynnpve](https://doi.org/10.17504/protocols.io.btynnpve))
- Recombinant CHMP2B (1 mg/ml; prepared as in [<https://www.protocols.io/view/purification-of-chmp2b-dg8c3zsw>])
-  Rabbit anti-CHMP2B antibody **Abcam Catalog #ab33174**
- CHMP2B buffer:

	A	B
	Tris	20 mM
	pH	7
	NaCl	150 mM
	beta-mercaptoethanol	2 mM

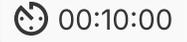
- PBS-T: 1X PBS pH 7.2 (Thermo Fisher Scientific cat. no. 20012068), 0.02% Tween-20
 PBS, pH 7.2 **Thermo Fisher Scientific Catalog #20012068**
- 4X NuPAGE LDS Sample Buffer (Thermo Fisher Scientific cat. no. NP0007) supplemented with 5% 2-mercaptoethanol  NUPAGE LDS sample buffer (4x) **Thermo Fisher Scientific Catalog #NP0007**
- Dynabeads Protein G for Immunoprecipitation (Invitrogen Cat. no. 10003D)
 Dynabeads[®] Protein G for Immunoprecipitation **Thermo Fisher Catalog #10003D**
- Magnetic tube rack (e.g. Invitrogen cat. no. 12321D)
 Magnetic Tube Rack for 1.5mL or 15mL tubes **Catalog #12321D**
- Pierce Rapid Gold BCA Protein Assay Kit (Thermo Fisher Scientific cat. no. A53225)
 Pierce[®] Rapid Gold BCA Protein Assay Kit **Thermo Fisher Catalog #A53225**
- Low bind 1.5 ml centrifuge tubes (Eppendorf cat. no. 0030108116)
 Protein LoBind Tubes, 1.5 mL **Eppendorf Catalog #0030108116**

Troubleshooting



Sample Preparation

30m

- 1 Thaw alpha-synuclein monomers, fibrils, and CHMP2B  . 
- 2 Centrifuge alpha-synuclein monomers and CHMP2B for  00:10:00 at  16000 x g, 4°C .  
- 3 During the centrifugation, Vortex the Dynabeads vial until re-suspended.  
- 4 Pipette  20 μL of beads into an Eppendorf tube along with  500 μL PBS-T. 
- 5 Place the tube in the magnetic stand to separate the beads from the solution.
- 6 Remove and discard the supernatant.
- 7 Add  2 μL of the anti-CHMP2B antibody along with  200 μL PBS-T into the tube. 
- 8 Incubate at  Room temperature , with rotation, for  00:20:00 to bind the antibody to beads.  
- 9 Once the centrifugation has ended, remove the supernatant and place the alpha-synuclein monomers and CHMP2B into new low bind tubes. 
- 10 Perform a Rapid BCA Gold Protein Assay on the alpha-synuclein monomers and CHMP2B to determine their concentrations.
- 11 Prepare 4 tubes:
 - 11.1 5 μM monomers/CHMP2B:

A	B
alpha-synuclein monomers	18.4 µg
CHMP2B	29.9 µg
PBS-T	250 µl

11.2 5 µM PFFs/CHMP2B:

A	B
alpha-synuclein PFFs	18.4 µg
CHMP2B	29.9 µg
PBS-T	250 µl

11.3 5 µM monomers alone:

A	B
alpha-synuclein monomers	18.4 µg
CHMP2B	equivalent volume of CHMP2B buffer
PBS-T	250 µl

11.4 5 µM PFFs alone:

A	B
alpha-synuclein PFFs	18.4 µg
CHMP2B	equivalent volume of CHMP2B buffer
PBS-T	250 µl

12 Remove  12.5 µL of each tube and add to a new tube to run as an input fraction.

13 Dilute the input fraction with an additional  12.5 μL of PBS-T, then add  25 μL NuPAGE LDS sample buffer and denature for  00:10:00 at  70 $^{\circ}\text{C}$.  10m 

Immunoprecipitation

14 Place the tube with the beads on the magnetic rack and remove the supernatant.

15 Wash the beads one time with 1000 PBS-T. 

16 Add the protein solutions to the tube with the beads and gently pipette to resuspend. 

17 Incubate with rotation at  Room temperature for  00:45:00 . 



18 Remove the tubes from the roller and place on the magnetic stand.

19 Remove the supernatant and keep in a separate tube to run as a flow-through fraction.

20 Take the tube with the beads off of the magnet. Add  1000 μL PBS-T and gently pipette to wash.  

21 Put back on magnetic stand, and remove and discard the supernatant.

22 Repeat the wash step 5 times. 

23 After your final wash, re-suspend the beads in  100 μL PBS-T and transfer to a clean tube.  

24 Remove supernatant with magnetic rack.

25 Elute using  25 μL NuPAGE LDS sample buffer and  25 μL PBS-T and incubate for  00:10:00 at  70 $^{\circ}\text{C}$.

10m



26 Add  12.5 μL of the flow-through fraction to  12.5 μL PBS-T and  25 μL NuPAGE LDS sample buffer and denature for  00:10:00 at  70 $^{\circ}\text{C}$.

10m



27 Remove the tubes containing the beads and place on the magnetic stand.

28 Remove the eluate and transfer to a new tube.