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Pulldown of protein aggregates with a biotinylated peptide

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We use this protocol and it's working

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Abstract

The purpose of this protocol is to test whether monomeric or aggregated versions of a protein can bind to a peptide. This is tested by performing a pulldown using an affinity reagent that consists of a biotinylated peptide conjugated to streptavidin beads. This protocol describes how to perform this pulldown with α-syn and Aβ42 monomers and aggregates as prey, but the same pulldown protocol could be extended to any prey of interest. The endpoint of this protocol is the production of input, flow-through, and eluate fractions from the pulldown, which can be analyzed by SDS-PAGE and/or immunoblot.

Materials

- Pierce Magnetic Streptavidin Beads
- Biotinylated peptide of interest
- Peptide Solubilization Buffer: 45% ethanol, 10% acetic acid, 1 mM DTT in ddH2O.
- 330 µM α-syn A53T monomers, prepared as in dx.doi.org/10.17504/protocols.io.btynnpve.
- 330 µM α-syn A53T aggregates (PFFs), prepared as in dx.doi.org/10.17504/protocols.io.btynnpve.
- Lyophilized Aβ42 protein, prepared exactly as in described in the “without urea” protocol in Linse 2020 (Section 3.6)

1. Alternatively, this protein can be bought, e.g. Thermo Fisher Scientific cat. no. 03-111

- Blocking Buffer:

PBS, pH 7.2 **Thermo Fisher Scientific Catalog #**20012068, 1% Triton™ X-100 Surfact-Amps™ Detergent Solution **Thermo Fisher Catalog #**85111, additional 113 mM NaCl (final concentration 250 mM), 3% BSA **Cell Signaling Technology Catalog #**9998S

- IP Buffer: PBS pH 7.2, 1% Triton X-100, additional 113 mM NaCl (final concentration 250 mM), 0.1% SDS, 1 mM DTT
- 500 mM NaCl in ddH2O.
- GE Buffer: 6M GuHCl, 20 mM sodium phosphate, pH 8.5
- Aβ42 Aggregation Buffer: 20mM sodium phosphate pH 8.5, 0.2 mM EDTA
- NUPAGE LDS sample buffer (4x) **Thermo Fisher Scientific Catalog #**NP0007 + 5% 2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250
- HU Buffer: 8 M urea, 5% SDS, 200 mM Tris-HCl pH 6.8, 1 mM EDTA, 0.01% bromophenol blue, 5% β-mercaptoethanol
- Formic Acid
- Superdex 75 Column (GE HealthCare cat. no. GE17-5174-01)
- FPLC Setup
- Microcentrifuge
- Magnetic tube rack
- Tube rotator
- Bioruptor Plus sonication system **Diagenode Catalog #**B01020001 (or equivalent)
- Nanodrop
- Thermomixer
- SpeedVac
- Protein LoBind 1.5mL microcentrifuge tubes **Eppendorf Catalog #**0030108116
- 96-well Half Area Black/Clear Flat Bottom Polystyrene (Corning cat. no. 3881)

Solubilization and Aggregation of Aβ42

1d

1 Equilibrate a Superdex 75 column with Aβ42 Aggregation Buffer.

2 Resuspend 500 µg of lyophilized Aβ42 in 700 µL GE Buffer.

3 Use an FPLC setup to run the solubilized Aβ42 through the Superdex 75 column, collecting the fractions in low bind tubes.

4 Check the fractions by [M] 205 nanomolar (nM) absorbance on a Nanodrop and pool the Aβ42-containing fractions.

5 Dilute the Aβ42 to [M] 10 micromolar (µM) in Aβ42 Aggregation Buffer.

6 Aliquot and snap freeze half of the sample to use later as Aβ42 monomers.

7 Place 80 µL in each well of a 96-well plate and incubate for 24:00:00 at 37 °C . 1d

8 Aliquot the aggregated material, flash freeze, and store at -80 °C .

Conjugation of the biotinylated peptide to Streptavidin resin (bait)

2h

9 Dissolve in Peptide Solubilization Buffer to an appropriate concentration.

9.1 In our case, we resuspend our peptide to 2 µL which was [M] 390.6 micromolar (µM) .

10 Add 25 µL of Pierce Magnetic Streptavidin Beads to a low bind tube and place on the magnetic rack.

10.1 Be sure to pipette with a cut pipette tip to avoid damaging the resin.

10.2 Prepare as twice as many tubes as prey proteins that will be pulled down. One tube will be for the normal pulldown and the other will be a no peptide control to assess background binding.

11 Wash the beads once with 1 mL IP Buffer.

12 Resuspend the beads in 400 µL IP Buffer.

13 Add 3.2 µL of the solubilized biotinylated peptide to the half of the tubes with beads.

14 To the other half add the same volume of Peptide Solubilization Buffer.

15 And rotate the tube for 01:00:00 at Room temperature to allow the biotinylated peptide to complex with the streptavidin. 1h

16 Wash the beads twice with 500 µL of [M] 500 millimolar (mM) NaCl, then once with 1 mL IP buffer.

17 Incubate the beads with rotation in 500 µL Blocking Buffer for 01:00:00 . 1h

18 Wash the beads once with IP Buffer.

Preparation of protein aggregates and monomeric controls (prey) for pulldown 31m 10s

19 Thaw the Aβ42 aggregates, α-syn monomers, and α-syn PFFs On ice .

20 Centrifuge the Aβ42 and α-syn monomers at 20000 x g, 4°C, 00:30:00 to remove any aggregated material. 30m

21 Remove the supernatant and place in a new tube.

22 Sonicate the α-syn PFFs in the Bioruptor for 5 cycles of 00:00:30 on and 00:00:30 off. 1m

23 Sonicate the Aβ42 aggregates for 5 cycles of 00:00:05 on and 00:00:05 off. 10s

24 Dilute each sample to [M] 2.5 micromolar (µM) in IP Buffer.

Pulldown 1h 0m 30s

25 Add 400 µL of [M] 2.5 micromolar (µM) Aβ42 monomers, Aβ42 aggregates, α-syn monomers, and α-syn to appropriate tubes.

25.1 Each sample should go into one tube with peptide-conjugated beads and into one peptide-free control tube.

25.2 Reserve a small amount of the [M] 2.5 micromolar (µM) sample to run as an input fraction.

26 Rotate the tubes for 01:00:00 at Room temperature, except for the Aβ42 monomers, which need to be incubated at 4 °C to prevent aggregation. 1h

27 Gently centrifuge the tubes (~ 50 x g, 00:00:30) to remove the resuspended beads from the cap area of the tube. 30s

28 Place the tube on the magnetic rack and set aside the supernatant to run as a flow-through fraction.

29 Using the magnetic rack, wash the beads three times with 500 µL IP Buffer.

29.1 On the last wash, transfer to a new tube.

Normal Elution (appropriate for α-syn)

10m

30 Replace the IP Buffer with 50 µL 4X NuPAGE LDS Sample Buffer + 5% β-mercaptoethanol.

31 Gently flick the tube to resuspend the resin.

32 Boil the tube for 00:10:00 at 95 °C.

10m

33 Use the magnetic rack to remove the supernatant, which will be the eluate fraction.

34 The input and flow-through fractions can now be denatured by boiling with the NuPAGE LDS Sample Buffer + β-mercaptoethanol (diluted 1:4).

Harsh Elution (appropriate for Aβ42)

1h 50m

35 Replace the IP Buffer with 100 µL formic acid.

36 Add 90 µL formic acid to 10 µL of the input and flow-through fractions.

37 Incubate at 19 °C for 00:40:00 with shaking at 500 rpm in a Thermomixer.

40m

38 Use the magnetic rack to remove the supernatant from the beads and place the supernatant in a new tube.

39 Evaporate the all fractions containing formic acid in a SpeedVac for 01:00:00 at 45 °C . 1h

40 Add 50 µL HU Buffer + 5% β-mercaptoethanol to the tubes and incubate for 00:10:00 at 60 °C shaking at 500 rpm in a thermomixer. 10m

Protocol references

1. Linse, S. (2020). Expression and Purification of Intrinsically Disordered Aβ42 Peptide and Setup of Reproducible Aggregation Kinetics Experiment. In: Kragelund, B.B., Skriver, K. (eds) Intrinsically Disordered Proteins. Methods in Molecular Biology, vol 2141. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0524-0_38