Mar 27, 2025

Purification of Recombinant Amyloid Beta Peptide (M1-42) from *Escherichia coli*

DOI
https://dx.doi.org/10.17504/protocols.io.3byl4z2k2vo5/v1

Patricia Yuste-Checa[1], Nadine Wischnewski[1], F Ulrich Hartl[1]

[1]Department of Cellular Biochemistry, Max Planck Institute of Biochemistry

Patricia Yuste-Checa
MPI Biochemistry

DOI: https://dx.doi.org/10.17504/protocols.io.3byl4z2k2vo5/v1

Protocol Citation: Patricia Yuste-Checa, Nadine Wischnewski, F Ulrich Hartl 2025. Purification of Recombinant Amyloid Beta Peptide (M1-42) from Escherichia coli. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4z2k2vo5/v1

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

Created: March 20, 2025

Last Modified: March 27, 2025

Protocol Integer ID: 125065

Keywords: ASAPCRN, purification of recombinant amyloid beta peptide, recombinant amyloid beta peptide, amyloid beta peptide, aβ, purification, escherichia coli, amino acid, amyloid beta

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000282

Abstract

This protocol is based on Linse S. *Methods Mol Biol*. (2020), section “3.6 Purification of Aβ(M1–40) or Aβ(M1–42) without urea” and details how to efficiently purify Amyloid beta peptide (amino acids 1-42) with a starting methionine (Aβ, M1-42) from *Escherichia coli* inclusion bodies.

Materials

Buffers

- **TE 7.5**:

	A	B
	Tris-HCl pH 7.5	10 mM
	EDTA	1 mM

- **TE 9.5**:

	A	B
	Tris-HCl pH 9.5	10 mM
	EDTA	1 mM

- **TE 8.5**:

	A	B
	Tris-HCl pH 8.5	10 mM
	EDTA	1 mM

- **TENa500**:

	A	B
	Tris-HCl pH 8.5	10 mM
	NaCl	500 mM
	EDTA	1 mM

- **Gu6**:

	A	B
	Guanidinium hydrochloride	6 M
	Sodium phosphate, pH 8.5	20 mM

- **AE**:

	A	B
	Ammonium acetate	20 mM
	EDTA, pH 8.5	0.2 mM

pET-Sac-Abeta(M1-42) **addgene Catalog #**71875

Transformation and Aβ (M1-42) Expression

1 Thaw RbCl-competent *Escherichia coli* BI21 cells (DE3) On ice .

2 Add 1 µL of pET-Sac-Abeta(M1-42) plasmid (Addgene plasmid # 71875) and incubate 00:30:00 On ice . 30m

3 Heat shock 00:00:45 at 42 °C . 45s

4 Incubate On ice 00:02:00 , then add 850 µL Lysogeny broth (LB) or Super Optimal broth with Catabolite repression (SOC) medium. 2m

5 Shake for 01:00:00 at 37 °C . 1h

6 Centrifuge for 3000 x g, 00:05:00 and remove most of the supernatant. 5m

7 Resuspend the pellet with the remaining supernatant and plate the bacteria on LB /Ampicillin agar plates and incubate Overnight at 37 °C.

8 **Prepare preculture**:

8.1 Pick 5 colonies and inoculate 13 mL LB/Ampicillin.

8.2 Shake at 37 °C for 4-6 h.

9 Take one clone and inoculate 200 mL LB/Ampicillin, shake Overnight at 37 °C .

10 Measure a 1:10 dilution of the overnight culture and inoculate 6 L of LB medium to an OD_{600} = 0.05.

11 Shake flasks at 90 rpm, 37°C until approx. OD_{600} = 0.4 (2.5 h).

12 Add isopropyl β-D-1-thiogalactopyranoside (IPTG) at final concentration of [M] 0.4 millimolar (mM) .

13 Shake flasks at 80 rpm, 37°C, 04:00:00 (OD_{600} = 1.3).

14 Centrifuge the bacterial culture at 4000 rpm, 4°C, 00:30:00 . Discard supernatant. 30m

15 Resuspend each Liter of main culture with 20 mL cold double-distilled water and pipet in 50 mL tubes.

16 Centrifuge again at 5000 x g, 4°C, 00:30:00 , remove supernatant and flash-freeze in liquid nitrogen for storage at -80 °C . 30m

Lysis

17 Thaw the cell pellets at Room temperature and resuspend them in 80 mL of TE 7.5 buffer supplemented with Complete EDTA-free protease inhibitor cocktail (Merck) and Sm DNase 50 µL .

18 Sonicate lysate On ice , 6 cycles 00:00:30 ON, 00:01:30 OFF (output 8, 53%).

19 Centrifuge lysate at 18000 x g, 4°C, 00:10:00 . 10m

20 Remove the supernatant (S1).

21 Resuspend the pellet in 80 mL of TE 7.5 buffer supplemented with Complete EDTA-free protease inhibitor cocktail (Merck) and Sm DNase 50 µL, until the sample is homogenous.

22 Sonicate lysate On ice, 6 cycles 00:00:30 ON, 00:01:30 OFF (output 8, 53%).

23 Centrifuge lysate at 18000 x g, 4°C, 00:10:00. 10m

24 Remove the supernatant (S2).

25 Resuspend the pellet in 80 mL of TE 7.5 buffer until the sample is homogenous.

26 Sonicate lysate On ice, 6 cycles 00:00:30 ON, 00:01:30 OFF (output 8, 53%).

27 Centrifuge lysate at 18000 x g, 4°C, 00:10:00. 10m

28 Remove the supernatant (S3).

29 Resuspend the pellet (inclusion bodies) using a glass rod and a magnetic stirrer with 80 mL of TE 9.5 buffer until the sample is homogenous.

30 Sonicate lysate On ice, 6 cycles 00:00:30 ON, 00:01:30 OFF (output 8, 53%).

31 Centrifuge lysate at 18000 x g, 4°C, 00:10:00. 10m

32 Collect the supernatant in a glass beaker. If the sample is turbid, filter through a 0.22 µm filter.

33 Adjust the pH to pH 8.5 with [M] 1 Mass Percent HCl (around 6 drops).

Diethylaminoethyl Cellulose (DEAE-C) Chromatography (Anion Exchange)

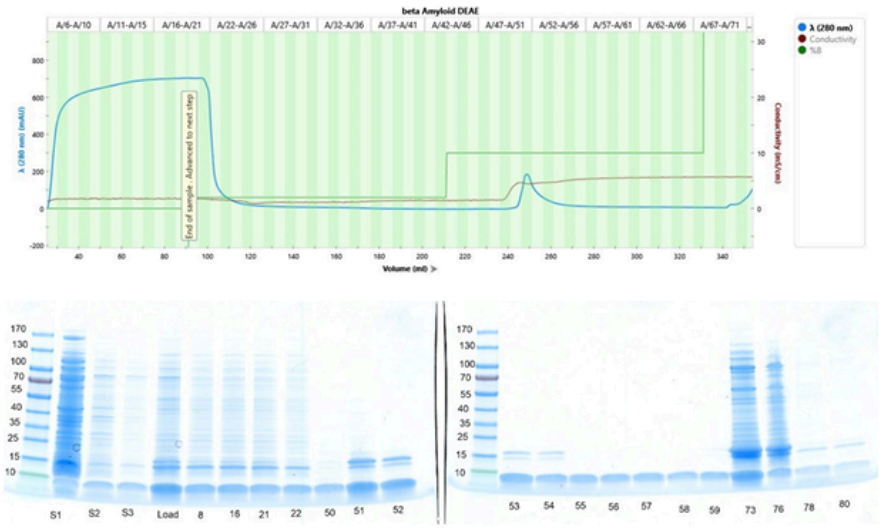
34 Equilibrate the DEAE-C column (Cytiva, GE17-0709-10) with 2 column volumes (CV, 30 mL) TE 8.5 buffer.

35 Load supernatant from inclusion bodies resuspension to the DEAE-C column.

36 Wash the DEAE-C column with 4 CV TE 8.5 buffer.

37 Elute Aβ (M1-42) with 4 CV 10% TENa500 buffer and collect elution fractions.

38 Analyze eluted fraction by SDS-PAGE and Coomassie blue staining.



DEAE-C chromatogram and SDS PAGE analysis. 5 µL sample + 5 µL 2x SDS sample loaded on a
4-20% SDS-PAGE.

Desalting and Lyophilization

39 Pool fractions containing Aβ (M1-42) (50-59).

40 In order to reduce the salt concentration, pass the eluted peptide through a HiPrep 26/10 desalting column equilibrated with the TE 8.5 buffer.

41 Pool the fraction containing Aβ (M1-42), and transfer the pool into a round bottom flask and freeze the sample while rotating in liquid nitrogen to cover the entire surface with ice.

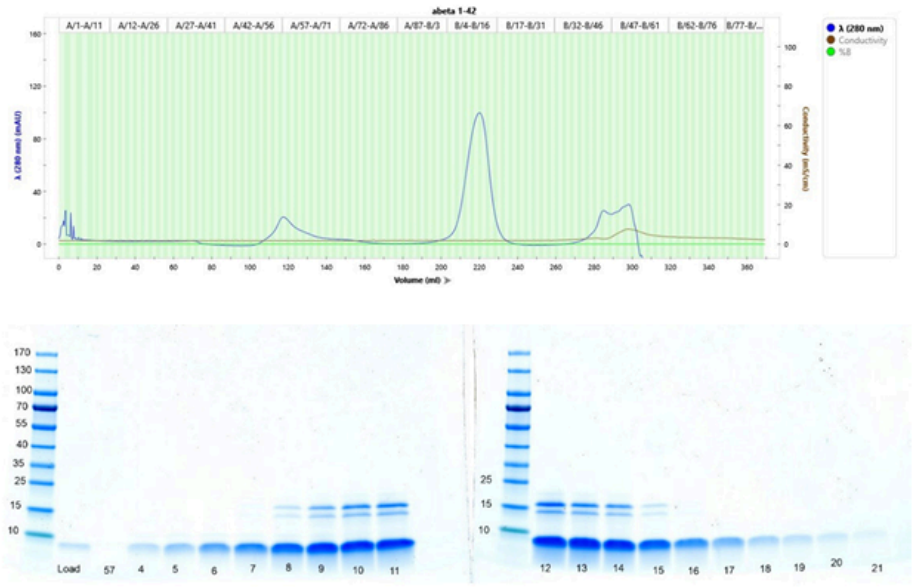
42 Lyophilize the frozen sample.

Size Exclusion Chromatography

43 Dissolve lyophilized Aβ (M1-42) to a final volume of 15 mL with Gu6 buffer.

44 Load the sample onto a Cytiva HiLoad 26/600 Superdex 75 pg SEC column previously equilibrated with AE buffer. Use AE as running buffer.

45 Analyze eluted fractions by SDS-PAGE and Coomassie blue staining.



Size exclusion chromatogram and SDS PAGE analysis. 5 µL sample + 5 µL 2x SDS sample dye loaded on a 4-20% SDS-PAGE.

46 Pool fractions containing Aβ (M1-42) and measure peptide concentration.

Note

NOTE: Aβ (M1-42) contains just one tyrosine and no tryptophan, therefore measure peptide concentration in a Nanodrop at absorbance 205 nm (A205).

47 Aliquot the peptide in 500 µg aliquots in 1.5 mL tubes and flash-freeze in liquid nitrogen.

48 Lyophilize the sample, seal each tube in plastic foil to avoid rehydration and storage at -80 °C .

Protocol references

Linse S. Expression and Purification of Intrinsically Disordered Aβ Peptide and Setup of Reproducible Aggregation Kinetics Experiment. Methods Mol Biol. 2020;2141:731-754. doi: 10.1007/978-1-0716-0524-0_38. PMID: 32696387.