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Purification of recombinant Tau Repeat Domain (TauRD) from Escherichia coli

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We use this protocol and it's working

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Abstract

This protocol details how to efficiently purify the recombinant Tau repeat domain from Escherichia coli.

Attachments

DOCX

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1.7MB

Guidelines

NOTE : This protocol was optimized for purification of the cysteine-free TauRD (Tau residues 244-371, C291A/P301L/C322A/V337M), but in principle any Tau isoform or mutant could be purified following this method if it is tagged with a His 6 -ubiquitin tag. If cysteines are present, reducing agent should be added to buffers to avoid the formation of disulphide bonds.

Materials

Terrific Broth (TB) media :

	A	B
	Yeast extract	24 g/L
	Tryptone	20 g/L
	Glycerol	4 mL/l
	KH2PO4	0.017 M
	K2HPO4	0.072 M

Lysis buffer :

	A	B
	PIPES-NaOH pH 6.5	50 mM
	NaCl	250 mM
	Imidazole	10 mM
	β-mercaptoethanol (β-ME)	2 mM

Ni-NTA high salt buffer :

	A	B
	PIPES-NaOH pH 6.5	50 mM
	NaCl	500 mM
	Imidazole	10 mM
	β-ME	2 mM

Ni-NTA wash buffer :

	A	B
	PIPES-NaOH pH 6.5	50 mM
	NaCl	250 mM
	Imidazole	50 mM
	β-ME	2 mM

Ni-NTA elution buffer :

	A	B
	PIPES-NaOH pH 6.5	50 mM
	NaCl	50mM
	Imidazole	250 mM
	β-ME	2 mM

PIPES buffer or Cation exchange Buffer A :

	A	B
	PIPES-NaOH pH 6.5	50 mM
	β-ME	2 mM

Cation exchange Buffer B :

	A	B
	PIPES-NaOH pH 6.5	50 mM
	NaCl	1 M
	β-ME	2 mM

TauRD expression

1d 4h 37m 45s

1 Thaw RbCl-competent Escherichia coli Bl21 cells (DE3) On ice .

2 Add 1 µL of pHUE-TauRD plasmid (His 6 -ubiquitin-TauRD) 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 00:05:00 at 3000 x g 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 . 8h

8 Prepare preculture: Scrap all colonies with the scraper and inoculate 25 mL - 50 mL LB/Ampicillin. Shake at 37 °C for 4- 06:00:00 . 6h

9 Measure OD_{600} of the preculture and inoculate two flasks with 1 L of TB media each to an
OD_{600} = 0.05.

10 Shake flasks at 37 °C until approx. OD_{600} = 0.5-0.8. (2 - 04:00:00) 4h

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

12 Shake flasks Overnight at 37 °C . 8h

13 Centrifuge bacterial culture at 4000 rpm for 01:00:00 . Discard supernatant. Cell pellets can be stored at -80 °C . 1h

Ni-NTA chromatography 1h 32m

14 Resuspend the cell pellets with lysis buffer (50 mL lysis buffer/ 2 L bacteria culture) supplemented with Complete EDTA-free protease inhibitor cocktail (Merck) and benzonase.

15 Add 1 undetermined lysozyme and incubate gently shaking for 00:30:00 at 4 °C . 30m

16 Sonicate lysate On ice , 5 cycles 00:00:30 ON, 00:01:30 OFF. 2m

17 Centrifuge lysate at 40000 x g 01:00:00 at 4 °C . 1h

18 Prepare Ni-NTA column by transferring 10 mL Ni-NTA resin slurry to a column (5 mL column bed). Wash Ni-NTA column with 10 column volumes (CV, 50 mL) water and equilibrate with 10 CV (50 mL) lysis buffer.

19 Load lysate supernatant to Ni-NTA column.

20 Wash Ni-NTA column with 10 CV (50 mL) high salt buffer and 10 CV (50 mL) wash buffer.

21 Elute His 6 -ubiquitin-TauRD with 20 mL elution buffer and collect everything.

> **Note**
>
> Prepacked or any other Ni column can be used for His 6 -ubiquitin-TauRD purification.

His 6 -ubiquitin cleavage

8h

22 Dilute eluted protein 1:5 with PIPES buffer to reduce the amount of salt (20 mL eluted protein + 80 mL PIPES buffer).

23 Incubate diluted His 6 -ubiquitin-TauRD protein with 0.5 mg Usp2 ubiquitin protease at 4 °C Overnight .

8h

> **Note**
>
> Dilution of eluted protein is not needed for protease cleavage but recommended to avoid protein precipitation during incubation. Salt dilution is needed for the next purification step, cation exchange chromatography.

Cation exchange chromatography

24 Load the cleavage mixture onto a Source S cation exchange column previously equilibrated with cation exchange buffer A.

25 Wash the column with 5 CV of cation exchange Buffer A.

26 Elute TauRD with a 0- [M] 500 millimolar (mM) linear NaCl gradient in [M] 50 millimolar (mM) PIPES-NaOH pH 6.5 , [M] 2 millimolar (mM) β-ME (0-50% gradient from cation exchange

buffer A to cation exchange buffer B over 10 CV).

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

Size exclusion chromatography

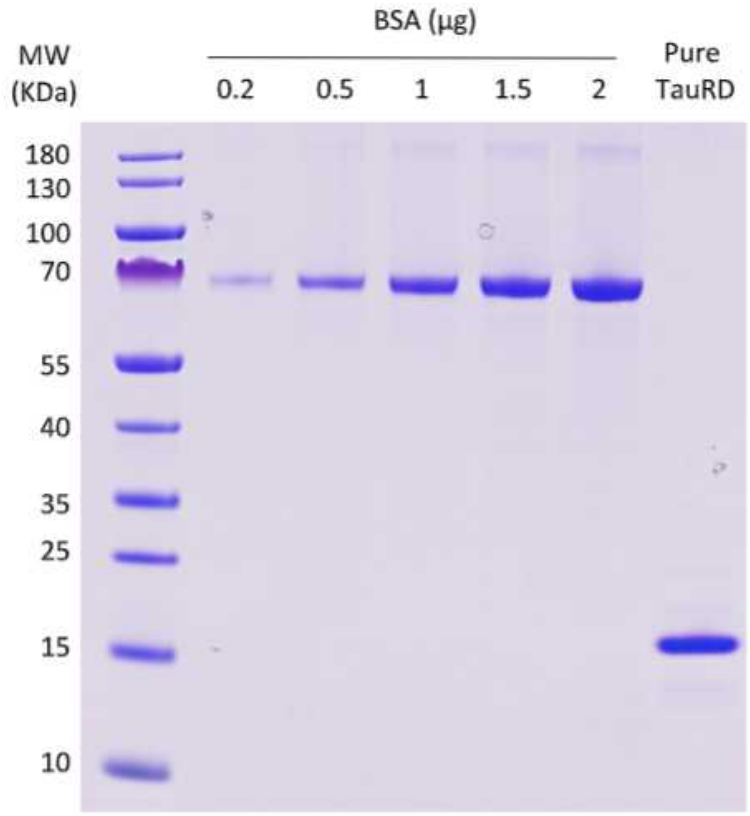
28 Load TauRD-containing fractions onto a Superdex-75 column previously equilibrated with PBS.

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

30 Pool fractions containing TauRD, aliquot and flash-freeze in liquid nitrogen for storage at -80 °C.

Note

- Due to the intrinsic disordered nature of Tau protein, the apparent size observed by size exclusion chromatography is larger than expected.
- TauRD protein contains few Tyr and Trp residues, and therefore the determination of pure protein concentration by OD_{280nm} is not reliable. We recommend to determine protein concentration of purified TauRD by BCA assay or Coomassie blue staining including a BSA standard curve. Rapid commercial Coomassie protein stain buffers are not recommended since sensitivity for the TauRD is very low. Standard Coomassie blue staining buffer should be used.



Note

- For TauRD thiol labelling, the mutation I260C could be introduced in the cysteine-free TauRD. The same purification protocol can be followed but [M] 1 millimolar (mM) tris(2-carboxyethyl)phosphine (TCEP) should be added to the size exclusion chromatography buffer in order to prevent the formation of disulfide bonds.
- Approximate yield: from 2 L of bacterial culture around 8 mg of pure TauRD are obtained.