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Expression and Purification of TailSpike 1 (TSP1) Protein from *E. coli*

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1 Works for me dx.doi.org/10.17504/protocols.io.pikdkcw

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ABSTRACT

This protocoll adds greater clarity and stepwise-discriptions regarding the purification of tailspike protein 1 (TSP1). The structure of TSP1 is 4OJ5.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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http://www.rcsb.org/structure/4OJ5

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11564

STEPS MATERIALS

NAME	CATALOG #	VENDOR
L-Arabinose	A-300	Gold Biotechnology
Benzonase® Nuclease	E1014 SIGMA	Sigma-aldrich
Lysozyme	12671-19-1	Sigma Aldrich
Imidazole	I5513	Sigma

NAME	CATALOG #	VENDOR
HisPur™ Ni-NTA Resin	88221	Thermo Fisher Scientific
Tris	RP-T60040	P212121

ABSTRACT

This protocoll adds greater clarity and stepwise-discriptions regarding the purification of tailspike protein 1 (TSP1). The structure of TSP1 is 4OJ5.

Prepare LB Broth and Grow Overnight Culture

1
1. Prepare 5-6L of LB broth in 4 liter baffled flasks. Sterilize for 20min. Cool in 37C with shaking < 80 rpm.
2. From a plate, inoculate a single colony into 300mL LB broth with 200ul carbenicillin (50mg/ml). Grow Overnight.

Cooling LB broth in shaker at 37C with minimal shaking reduces lag time when inoculating cultures the following morning.

Inoculate, Grow and Induce

2
1. Inoculate the 4L, baffled flasks containing LB broth with 20-50mL of the overnight culture. Adjust shaking speed to 180 rpm
2. Inoculate in the afternoon such that OD_{600} is between 0.8-1.0 before you leave. ABout 30 min before, cool incubator down to room temperature (21-25C)
3. Induce with 0.10% arabinose (10g in 1L).
4. Grow overnight at RT until 9a or 10a the following morning.

Do not add antibiotics to the broth in the flasks.
A 4-hr induction may also be done with 0.25% arabinose, but the yield will not be as high (about 1-2mg/L).
Overnight induction yield at 0.10% will be between 4-8mg/L.

L-Arabinose
by Gold Biotechnology
Catalog #: A-300

Purify TSP1

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1. Spin cultures down in 1L bottles between 4500-5000xg for 20 min using Beckman Coulter rotor JLA 8.1 or 9.1.
2. Resuspend culture in 1x PBS pH 7.4 with 10-20mM imidazole (MW 68.1) in 25-32mL. Transfer to 50mL conical tube.
3. Place conical tube in 60C water bath for 15 min. Cool to RT.
4. Add 5-10mg lysoszyme. Add 20ul Benzonase. Invert to mix.
5. Lyse cells using french press or other method e.g. sonication
6. Transfer lysate into 30mL round-bottom centrifuge tubes and spin at 20,000xg for 20-30min. Decant into new tubes.
7. Add 1mL resuspended Ni-NTA resin for each liter of induced culture to lysate.
8. Incubate with end/end rotation for 30 min.
9. Pellet resin by centrifuging for 5 min at 4000-5000xg. Carefully decant. Do not allow resin to be decanted.
10. Combine resin into single 50mL conical tube. Wash with 1x PBS and 20-40mM imidazole for 4-5, 50 mL column volumes.
11. After final wash, combine all resin into 15mL conical tube. Wash 1 CV. Centrifuge.
12. Carefully remove supernatant with electronic pipetter and serological pipette.
13. Add 2mL elution buffer (PBS 300-500mM imidazole).
14. Incubate with end/end rotation for 5-20 min.
15. Centrifuge. Collect supernatant in new tube, careful not to disturb resin.
16. Repeat 3 times, each with 2mL.

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17. Quantify protein concentration using spectrophotometric or other technique.

After resuspension in PBS and transfer to 50mL conical tubes, the samples may be placed in -20C freezer overnight. Do not add benzoase or lysozyme before freezing.

TSP1 is a stable trimer and steps may be carried out at room temperature.

Incubating resuspended pellets at 60C for 15 min helps lyse cells and deactivate proteases.

Benzonase® Nuclease
by Sigma-aldrich
Catalog #: E1014 SIGMA

Lysozyme
by Sigma Aldrich
Catalog #: 12671-19-1

Imidazole
by Sigma
Catalog #: I5513

HisPur™ Ni-NTA Resin
by Thermo Fisher Scientific
Catalog #: 88221

Dialyze TSP1 and Purify on Mono Q Column

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1. In 5 L container, add 20mM Tris pH10 (MW:121.1) and 20mM NaCl (MW:58.4).
2. Filter protein through 0.2uM filter.
3. Dialyze overnight using casettes or dialysis bag.
4. The following morning, concentrate in 100,000 MWCO protein concentrator to about 500 ul total volume.
5. From the dialysis buffer, pour 1 L into separate container. Adjust to 300mM NaCl. Degas.
6. Perform pump wash on pump B with 300mM solution. This will be the elution buffer on mono Q column (anion exchange column).
7. Perform protein loading, washing and purification according to manufacturer's protocol.
8. TSP1 should elute from mono Q column in single peak.
9. Use protein concentrators to concentrate eluted TSP1 to desired concentration.
10. Dialyze overnight in 1x PBS supplemented with 10% glycerol.
11. Adjust concentration to 3-4mg/ml. Use liquid nitrogen to snap-freeze tubes in 200ul aliquots.
12. Store tubes containing TSP1 protein in -80C freezer.

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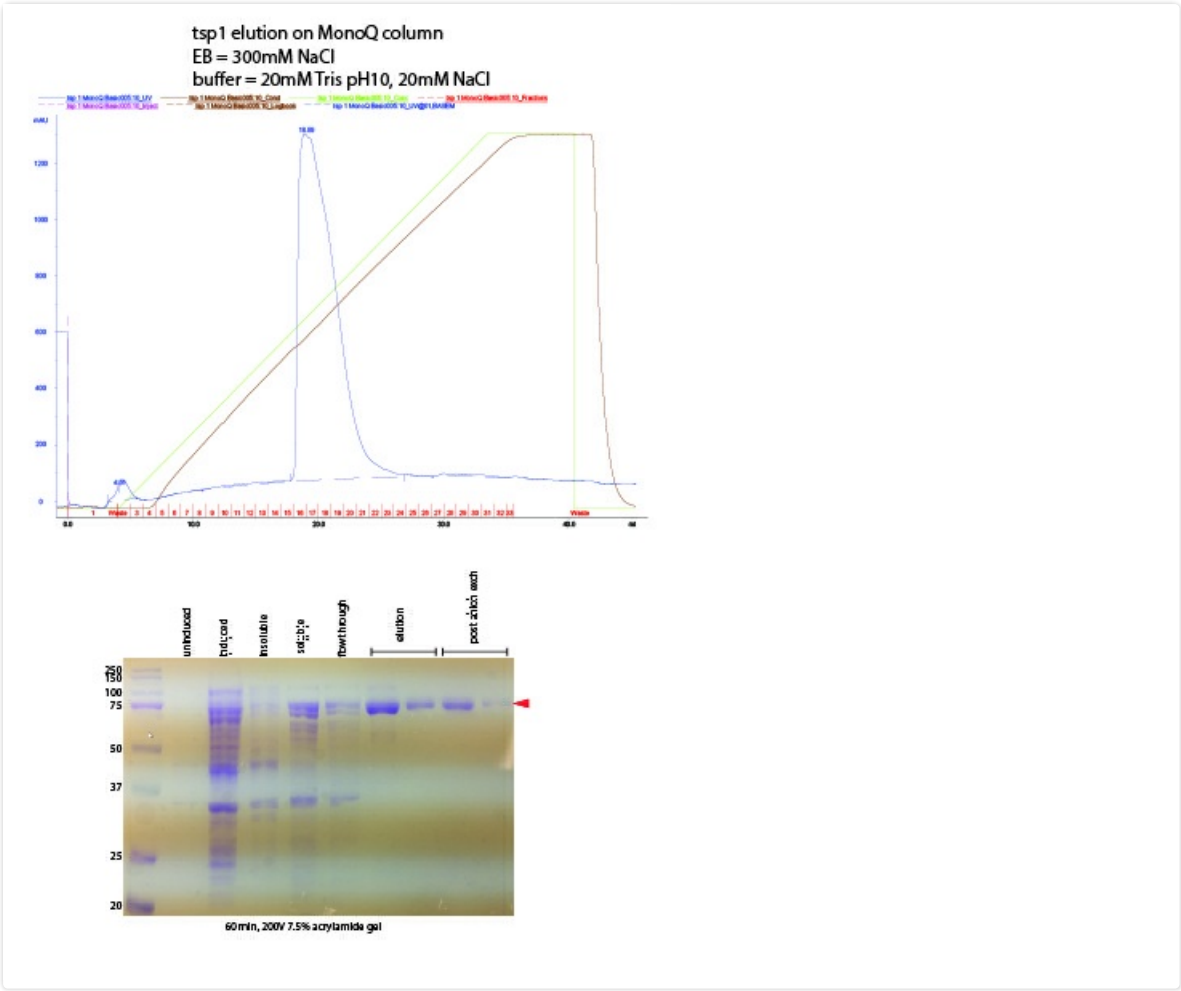

I add Tris powder to 5L container (121.14*5*0.02 = 12.11g) and not buffered Tris solution. The unbuffered Tris is usually around pH10. It's not an ideal buffer in this range but that's okay. pH needs to be about 2 pH units higher than TSP1 isoelectric point around 7.

Tris

by P212121

Catalog #: RP-T60040

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