Protocol for Purification of CDL21L1-179 endolysin in *E.coli* BL21 strain.
Code: LysP

Materials

- Ni-NTA resin
- Ultrapure water
- 10 mM Imidazole Buffer:
  - 300 mM NaCl
  - 50 mM NaH2PO4
  - 10 mM Imidazole
- 20 mM Imidazole Buffer:
  - 300 mM NaCl
  - 50 mM NaH2PO4
  - 20 mM Imidazole
- 500 mM Imidazole Buffer:
  - 300 mM NaCl
  - 50 mM NaH2PO4
  - 500 mM Imidazole
- Centrifuge
- Affinity chromatography column
- Noria shaker

Procedure

Ni-NTA resin wash

1. For a 400 mL culture, transfer 2 lysin supernatant volumes of Ni-NTA resin to a 1.5 mL tube. Centrifuge for 1 min at 500 rpm.
2. Remove the upper phase (400 uL) and add 400 uL of ultrapure water. Centrifuge with the same conditions, removing the upper phase at the end. Repeat the process two more times.
3. Do the step 3 but use 10 mM Imidazole Buffer instead of ultrapure water.
4. Resuspend the resin in 400 uL of 10 mM Imidazole Buffer.

Incubation

1. Combine the washed resin with the lysin supernatant extract in a 50 mL falcon tube.
2. Incubate for 3 hours in a noria shaker at 4°C.

Purification by Affinity Chromatography

1. Decant the lysin with the resin in an affinity chromatography column and collect the flow through in a falcon labeled as “Non Bound”.
2. Repeat step 1 with the flow through and collect it in the same falcon.

3. Wash the column with 15 resin volumes of 10 mM Imidazole Buffer and collect the flow through in a falcon labeled as “Column Wash 1”.
4. Wash the column with 15 resin volumes of 20 mM Imidazole Buffer and collect the flow through in a falcon labeled as “Column Wash 2”.
5. Elute the protein with 10 resin volumes of 500 mM Imidazole Buffer and collect the flow through in eppendorfs labeled as “Elution”.
6. Run a SDS-PAGE to confirm the presence of the purified protein.

References

Dunne, M., Mertens, H. D. T., Garefalaki, V., Jeffries, C.M. et al. (2014). The CD27L and CTP1L Endolysins Targeting Clostridia Contain a Built-in Trigger and Release Factor. PLoS Pathog 10(7): e1004228. doi:10.1371/journal.ppat.1004228