Ni-NTA agarose protein purification

Aim of the experiment

The purpose of this experiment is to purify the Cas13 protein, which has a 6×His tag and can be purified with Ni-NTA agarose. The whole experiment process should be carried out at 4℃ to prevent protein denaturation.

Materials

instrument:

- Vortex oscillator
- Ultrasonic disruptor
- Rotating incubator QB-328
- Microplate reader

Reagent:

- Phosphate Buffered Saline（1×）(PBS Buffer)
- Phenylmethyl sulfonyl fluoride （PMSF）
- Tris-HCl
- NaCl
- Tris(2-carboxyethyl)phosphine (TCEP）
- Imidazole
- Ultra-pure water
- 20% ethanol
- rTEV protease
- SUMO protease
- 50 mL column
- Ni-NTA Agarose
- Centrifugal filter

- Ultrafiltration tube
- BCA Protein Quantitation Assay Kit
- Buffer：

Table 1：Washing Buffer A

Reagent	concentration
Tris-HCl pH 8.0	50 mM
NaCl	30 mM
TCEP	1mM
Imidazole	10 mM
Constant volume to 1L，and degas ultrasonically	

Table 2：Washing Buffer B

Reagent	concentration
Tris-HCl pH 8.0	50 mM
NaCl	30 mM
TCEP	1mM
Imidazole	50 mM
Constant volume to 1L，and degas ultrasonically	

Table 3：Elution Buffer

Reagent	concentration
Tris-HCl pH 8.0	50 mM
NaCl	30 mM
TCEP	1mM
Imidazole	250 mM

Constant volume to 1L， and degas ultrasonically

Table 4： Washing Buffer C

Reagent	concentration
Tris-HCl pH 8.0	50 mM
NaCl	30 mM
TCEP	1mM
Imidazole	500 mM
Constant volume to 1L， and degas ultrasonically	

Table 5： PB Buffer

Reagent	concentration
$Na_2HPO_4 \cdot 12H_2O$	50 mM
$NaH_2PO_4 \cdot 2H_2O$	50 mM
Constant volume to 1L	

Procedure

1. Mix the thawed protein thawed cell pellet with PBS buffer（1×） at a ratio of 10g/L, suspend it with a vortex shaker and add 0.5 mM PMSF.

2. Lyse cells using ultrasound (3s pulse, 30 % amplitude, 8s pause).

3. Centrifuge the crushing liquid for 10 minutes at 4℃, 12000rpm.

4. Transfer the supernatant into a clean tube.

5. Aspirate the 20% ethanol in Ni-NTA agarose, and wash Ni-NTA agarose repeatedly with PBS buffer（1×）.

6. For every 8 ml lysate, pipette 1 ml Ni-NTA agarose into a 10 ml tube.

7. Place the test tube on a rotating incubator and incubate at 4°C for 90 minutes to make the protein and Ni-NTA agarose fully bound.

8. Load the mixture onto the column and remove the column's bottom cap and collect flow-through in a 50 ml tube, which is placed in ice,until the mixture is completely loaded.

9. Wash with 200ml Washing buffer A (table 1) and collect the flow-through with a 1.5ml EP tube (only one tube is sufficient),and mark the tube as "10mM".

10. Wash with 200ml Washing buffer B (table 2) and collect the flow-through with a 1.5ml tube (only one tube is sufficient),and mark the tube as "50mM"

11. Elute the protein with 1ml elution buffer (table 3) and collect the flow-through with a 1.5ml tube.

12. Repeat the above steps 10 times and mark the tubes as "250-1 to 250-10".

13. Wash with Washing buffer C (table 4), containing 500mM imidazole can clean the Ni-NTA agarose completely.

14. Finally, wash with ddH2O and 20% ethanol successively, and store the Ni-NTA agarose in 20% ethanol.

15. Analyze the collected flow-through sequentially in a SDS-PAGE.

16. Load the purified proteins in a dialysis membrane and add an appropriate amount of rTEV protease (for Lba) or SUMO protease (for Lwa、Psm and Cca) to cleave off the His-MBP tag or the His-SUMO tag respectively.

17. Dialyse the sample in a large volume of PB Buffer (table 5) overnight at 4℃.

18. Get rid of the cleaved off His-SUMO tag by repeating Ni-NTA agarose purification. This time however, the desired protein does not bind to the Ni-NTA agarose and can be collected in the first flow-through. Also elute the bound proteins to clean the Ni-NTA agarose for further use and also for SDS-PAGE analysis.

19. Analyze the collected flow-through sequentially in a SDS-PAGE.

20. Combine all the flow-through fluids containing Cas13 protein.Up-concentrate the sample to 3ml with a centrifugal filter (MWCO: 10 kDa) at 4500 rcf.

21. Check purity in a SDS-PAGE.

22. Measure the concentration via BCA Protein Quantitation Assay Kit.

23. Store the protein at -80℃.