Äkta Start protein purification

Aim of the experiment

The purpose of this experiment is to purify Cas13a Lwa, Lba and Cas13b Psm, Cca with Äkta Start protein purifier. All steps of this experiment were performed at 4°C to prevent protein degradation.

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
- ddH2O
- 20% ethanol
- rTEV protease
- SUMO protease
- 50 mL column
- Ni-NTA Agarose
- Centrifugal filter
- Ultrafiltration tube
- BCA Protein Quantitation Assay Kit

- Buffer

Table 1：Buffer A

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

Table 2：Buffer B

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 3：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. Wash the column with ddH2O at a flow rate of 5ml/min (wash on both sides of buffer A and buffer B by 50%)
6. 100% Buffer B to clean the column.

7. Equilibrate the column with 1% Buffer B (5mM Imidazole).

8. Load the protein solution on the column with a flow rate of 0.5ml/min (the flow rate is slower, which makes the protein bind to the column).

9. After loading the sample, clean with 1% Buffer B at a flow rate of 2ml/min and collect the flow-through with a 1.5ml EP tube.

10. Wash the column with 5% Buffer B (25 mM IM), 10% Buffer B (50 mM IM), 20% Buffer B (100 mM IM), 30% Buffer B (150 mM IM), 50% Buffer B (250 mM IM),and collect the flow-through with 50 ml tubes respectively.

11. Finally, wash the column with 100% BufferB (500mM IM), ddH2O, and 20% ethanol.

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

13. 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.

14. Dialyse the sample in a large volume of PB Buffer (table 3) overnight at 4℃.

15. 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.

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

17. 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.

18. Check purity in a SDS-PAGE.

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

20. Store the protein at -80℃.