12.10.2020

3 * 4 L 2YT medium:

64g tryptone

40g yeast

20g NaCl

Purification of the pellet from 27.08. (BL21 Ba from 4 L)

- Cell disruption see protocol
- 1st pillar see protocol

Preparation of the protease buffer 1 L

Tris: 6.057 g

EDTA: m 0.292 g

DTT: 0.15 g

NaCl: 8.766 g

Transformation see protocol

- With AD494 competent cells
- With the purchased Ba plasmid
- Plating on LB plates with Kan and Amp

14.10.2020

Phosphate buffer pH7

- Ba BL21 overnight

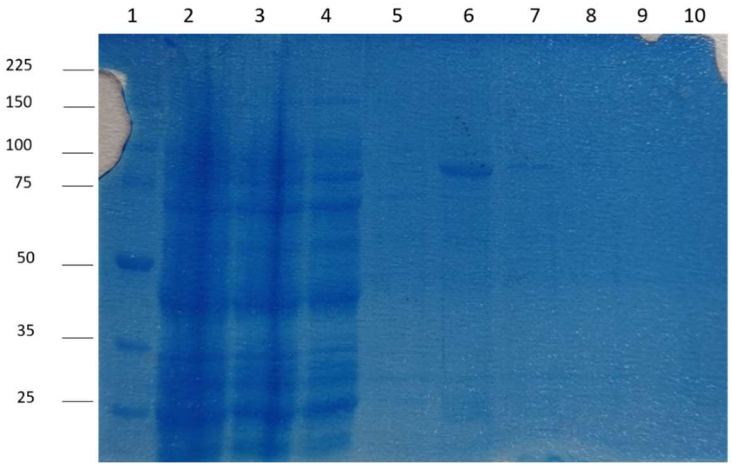
Overnight from AD494

- 70 ml in 1 l flask. With 21 µl Kan and 35 µl Amp.

Purification Ba BL21

- Phosphate buffer pH5,5 1,5h
- Phosphate buffer pH4 1,5h
- concentrate see protocol

E. coli BL21 (DE3) Ba pGEX-6P-1 with $CuSO_4$ medium: 2YT; 17°C overnight, 1st column



1: marker

2: before induction sample

3: after induction sample (3h, 30°C)

4: lysate

5: supernatant

6: wash

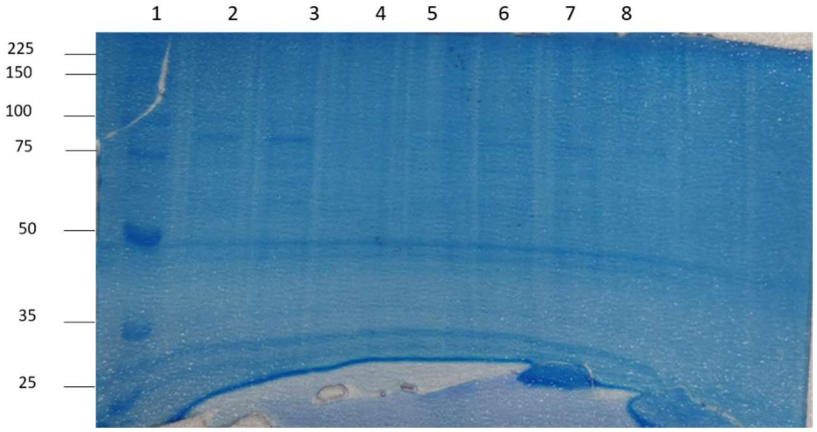
7: eluate 1

8: eluate 2

9: eluate 4

10: eluate 6

E. coli BL21 (DE3) Ba pGEX-6P-1 with $CuSO_4$ medium: 2YT; 17°C overnight, 2nd column



1: marker

2: protein before dialysis

3: protein after dialysis

4: D1

5: D2

6: D3

7: D4

8: eluate