

15.10.2020

Production of AD494

- A total of 6 liters -> 1 L per 5 L flask with 300 µl can and 500 µl amp and 100 µl copper sulfate.
- Add overnight liquid culture until the OD is set to approx. 0.03.
- Incubated at 37°C until OD reaches 0.5 (2 h, 40 min)
- Production at 17°C overnight

16.10.2020

Cell harvest see protocol

19.10.2020

Buffer preparation:

- Elution buffer:
Tris-HCl pH8: 25 ml
Glutathione: 0.076 g
- Preparation of the protease buffer 1L
Tris: 6.057 g
EDTA: m 0.292 g
DTT: 0.15 g
NaCl: 8.766 g

For digestion and purification of the AD494 Ba pellets (harvested on October 16 and stored at -20°C)
see protocol

- Adding PBS to resuspend
- AD494 Ba: fill up to the 10 ml mark

Dialysis see protocol

- Add 10 µl PreScission Protease each time

1st pillar see protocol

20.10.2020

Buffer preparation:

- Phosphate buffer:
Na₂HPO₄: total 2 L -> 71.196 g at a final concentration of 0.2 M.
Citric Acid: total 1.5 L -> 31.521 g at a final concentration of 0.1 M.

pH 7: 823.5 ml Na₂HPO₄
176.5 ml Citric Acid

pH 5.5: 568.7 ml Na₂HPO₄

431.2 ml of citric acid

pH 4: 385.5 ml Na₂HPO₄
614.5 ml Citric Acid

- Elution buffer:
Tris-HCl pH 8: 15 ml
Glutathione: 0.046 g

2nd pillar of AD494 Ba see protocol

Dialysis see protocol

- Dialysis in phosphate buffer pH 7

Test expression induced by AD494 Ba with different IPTG concentrations

- 6 times 50 ml in 500 ml flasks
- Add kanamycin and ampicillin
- Add 500 µl of the overnight culture

Overnight culture OD (1:10 dilution): 0,201

flask	Start OD	OD pre-induction (after 2:40 h)
1	0,019	0,653
2	0,029	0,695
3	0,010	0,637
4	0,027	0,625
5	0,009	0,676
6	0,029	0,666

Each flask is subsequently induced with a different concentration of IPTG

flask	Volume IPTG [µl]	concentraion IPTG [mM]
1	12,5	0,25
2	18,75	0,375
3	25	0,5
4	50	1
5	75	1,5
6	125	2,5

- Take a 1 ml sample from each flask
- Production overnight at 17 ° C

21.20.2020

finish dialysis of AD494 Ba from pH 7

- Dialyze in pH 5.5 for 1.5 h
- Dialyze in pH 4 for 1.5 h

Concentration of AD494 Ba pH 4 see protocol

→ 37.41 μM at 200 μl

OD of the test expression of AD494 Ba overnight at 17 ° C

flask	dilution	OD measured	OD calculated
1	1:10	0,365	3,65
2	1:10	0,350	3,5
3	1:10	0,301	3,01
4	1:10	0,260	2,6
5	1:10	0,371	3,71
6	1:10	0,326	3,26

- Take a 1 ml sample from each flask

Prepare the samples for loading the gel

- Pre-induction samples are not digested:

flask	water [μl]	SDS [μl]
1	36,73	12,24
2	39,09	13,03
3	35,83	11,94
4	35,15	11,71
5	38,025	12,675
6	37,46	12,48

- Overnight samples are digested see protocol
- Dissolve the pellet in PBS

flask	PBS [μl]
1	182,5
2	175
3	150,5
4	130
5	185,5
6	163

- Shock freeze samples in dry ice and thaw in 37°C incubator
- 15 min ultrasonic bath
- Freeze samples again in dry ice and in a 37°C incubator

- Remove the supernatant after centrifugation
- Add 10 μl water and 10 μl 4 x SDS sample buffer to 20 μl of the supernatant
- Dissolve the pellet in PBS

flask	PBS [μl]
1	91,3
2	87,5

3	75,3
4	65
5	92,8
6	81,5

loading gel:

Gel 1:

marker	VI 1	supernatant 1	pellet 1	VI 2	supernatant 2	pellet 2	VI 3	supernatant 3	pellet 3
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Gel 2:

marker	VI 4	supernatant 4	pellet 4	VI 5	supernatant 5	pellet 5	VI 6	supernatant 6	pellet 6
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SDS-PAGE see protocol

Western blot see protocol

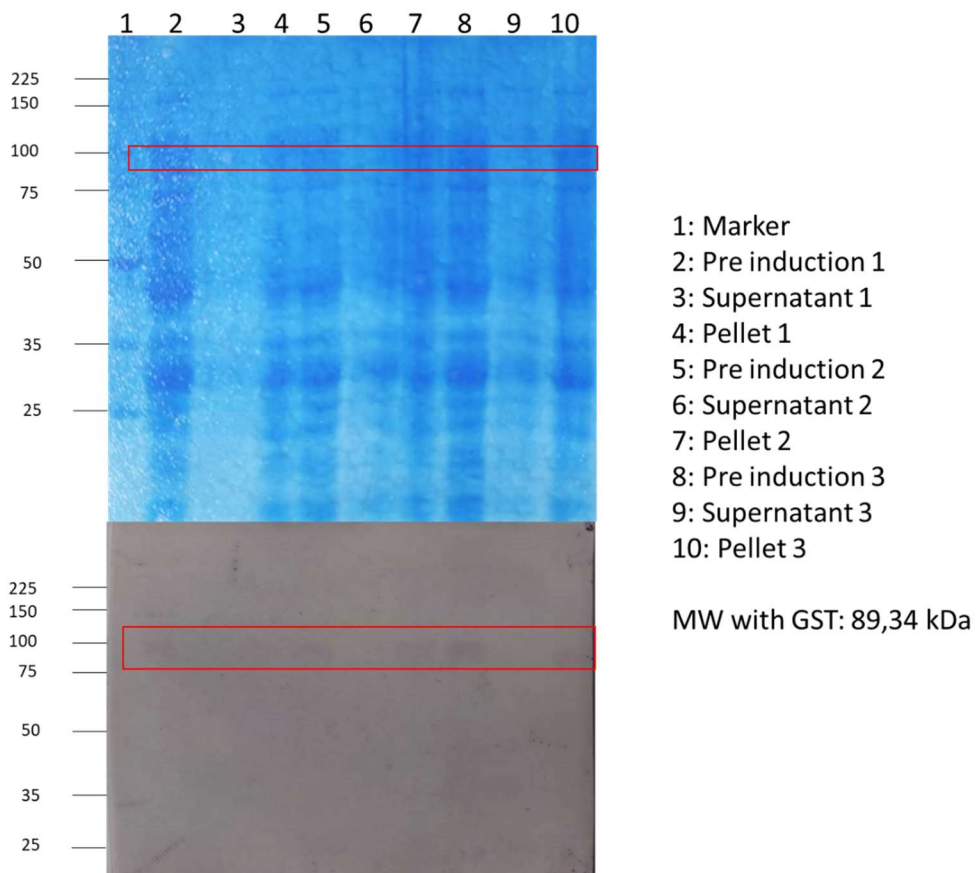
- Allow the blocking buffer to incubate at 4 ° C overnight

22.10.2020

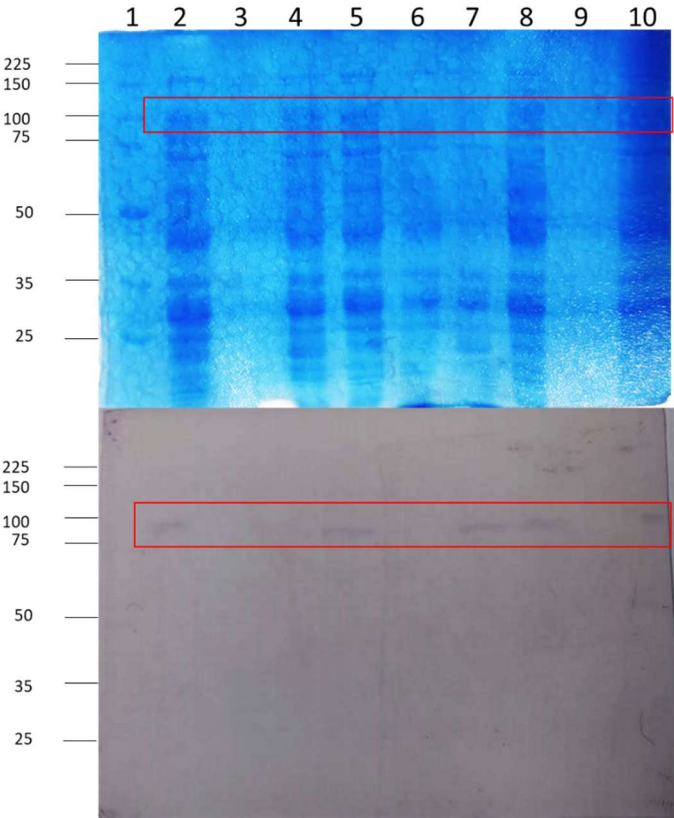
Western blot detection see protocol

- With BCIP and NBT

E.coli AD494 (DE3) Ba pGEX-6P-1 with CuSO₄ medium: 2YT; overnight; 17°C 1. gel



E.coli AD494 (DE3) Ba pGEX-6P-1 with CuSO₄ medium: 2YT; overnight; 17°C 2. gel



- 1: Marker
 - 2: Pre induction 1
 - 3: Supernatant 1
 - 4: Pellet 1
 - 5: Pre induction 2
 - 6: Supernatant 2
 - 7: Pellet 2
 - 8: Pre induction 3
 - 9: Supernatant 3
 - 10: Pellet 3
- MW with GST: 89,34 kDa