Summary of Results from Group 14 Lactase Downstream Processing

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Group 14 + presentation of group members

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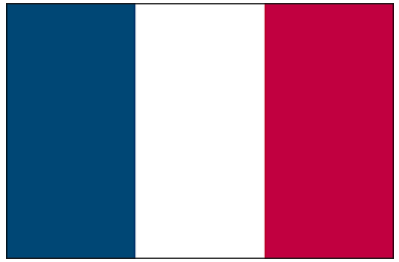

Evaluation Steps of our Experiment

1. **PARTICLE FREE LYSATE – o-NPG-test (enzymatic activity assay)**
2. ANION EXCHANGE CHROMATOGRAPHY (AEXC) – o-NPG + photometer (enzymatic activity assay)
3. SDS – PAGE – gel + calibration line (purity, molecular weight – lactase)
4. RAW MILK TEST – colour change (conc. of glucose)
5. EVALUATION

O-NPG-TEST of 1:10 diluted Particle Free Lysate

Test 50 µL supernatant + 500 µL o-NPG-solution.

- **Observation:** The color of the Particle Free Lysate was yellow
- **Evaluation:** $\beta(1\rightarrow4)$ bond was broken down
- **Conclusion:** lactase is present.

$\rightarrow$ We can start AEXC.

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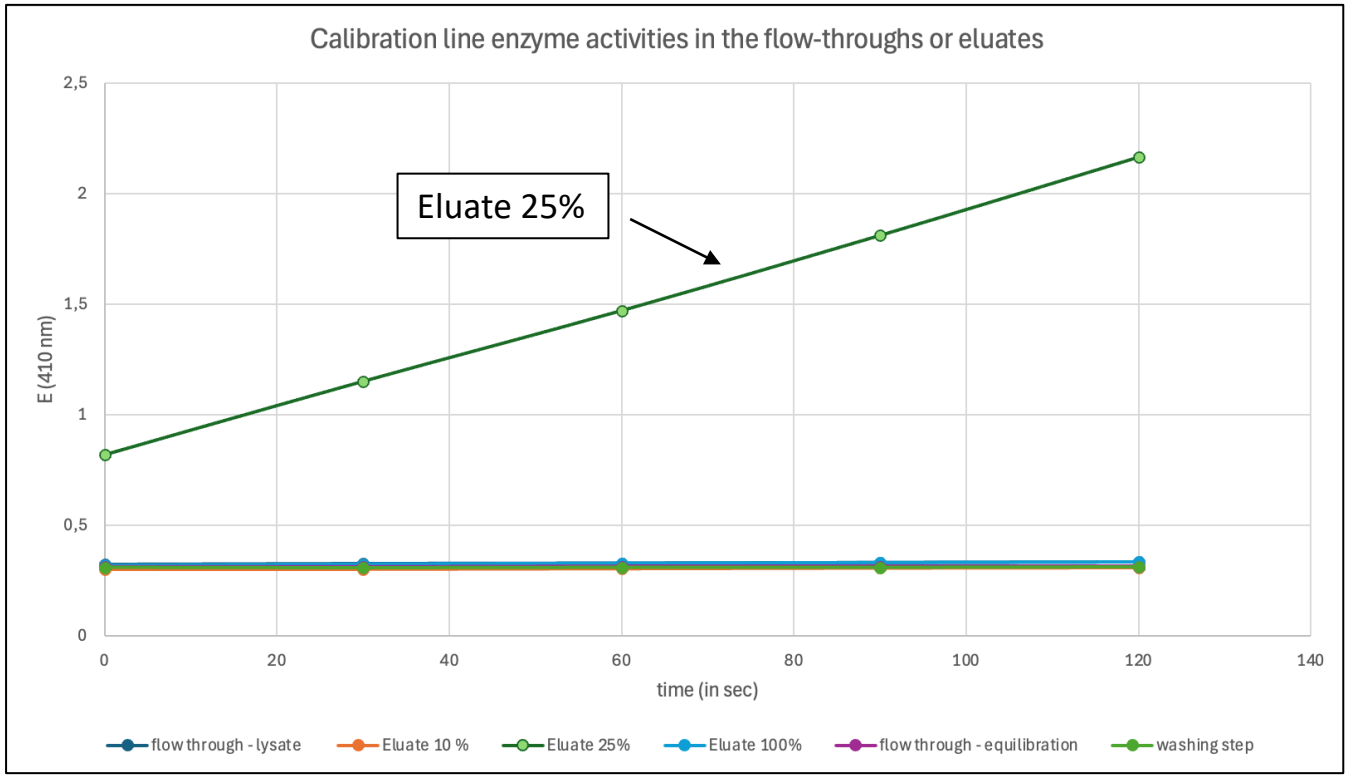
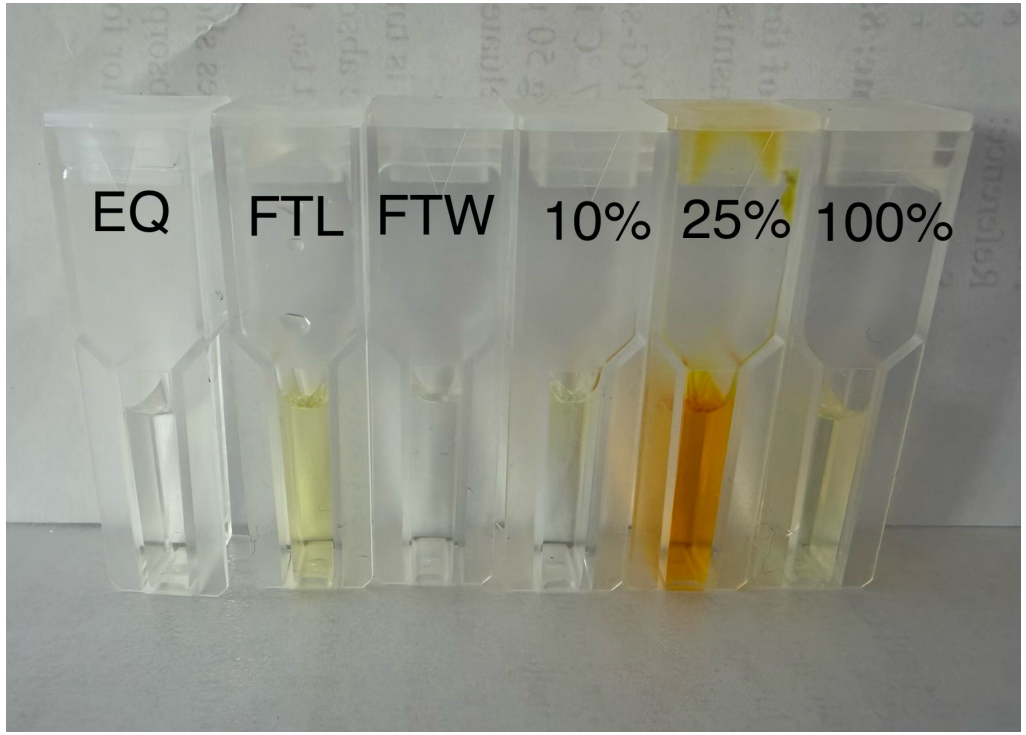
ANION EXCHANGE CHROMATOGRAPHY

O-NPG-test measured by photometer – enzymatic activity measurement

Test 50 µL eluate + 800 µL o-NPG-solution.

- **Observation:** We could observe that the Eluate 25% has the highest absorbance and is increasing
- **Evaluation:** Our observation tells us that the Eluate 25% has the most enzyme activity since the absorbance is increasing because we get more of the ortho-nitrophenol.
- **Conclusion:** The 25% buffer was the most optimal for the lactase to elute of the ones we used.





ONPG-Activity Assay of Lactase

time	0	30	60	90	120
flow through - equilibration	0,312	0,312	0,313	0,313	0,313
flow through - lysate	0,322	0,326	0,328	0,331	0,334
washing step	0,309	0,308	0,309	0,309	0,31
Eluate 10 %	0,301	0,301	0,304	0,307	0,309
Eluate 25%	0,818	1,15	1,47	1,81	2,164
Eluate 100%	0,318	0,323	0,327	0,33	0,333

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SDS - PAGE

M: MARKER

Lys: lysate (dilution factor 10)

FTL: loading flow through

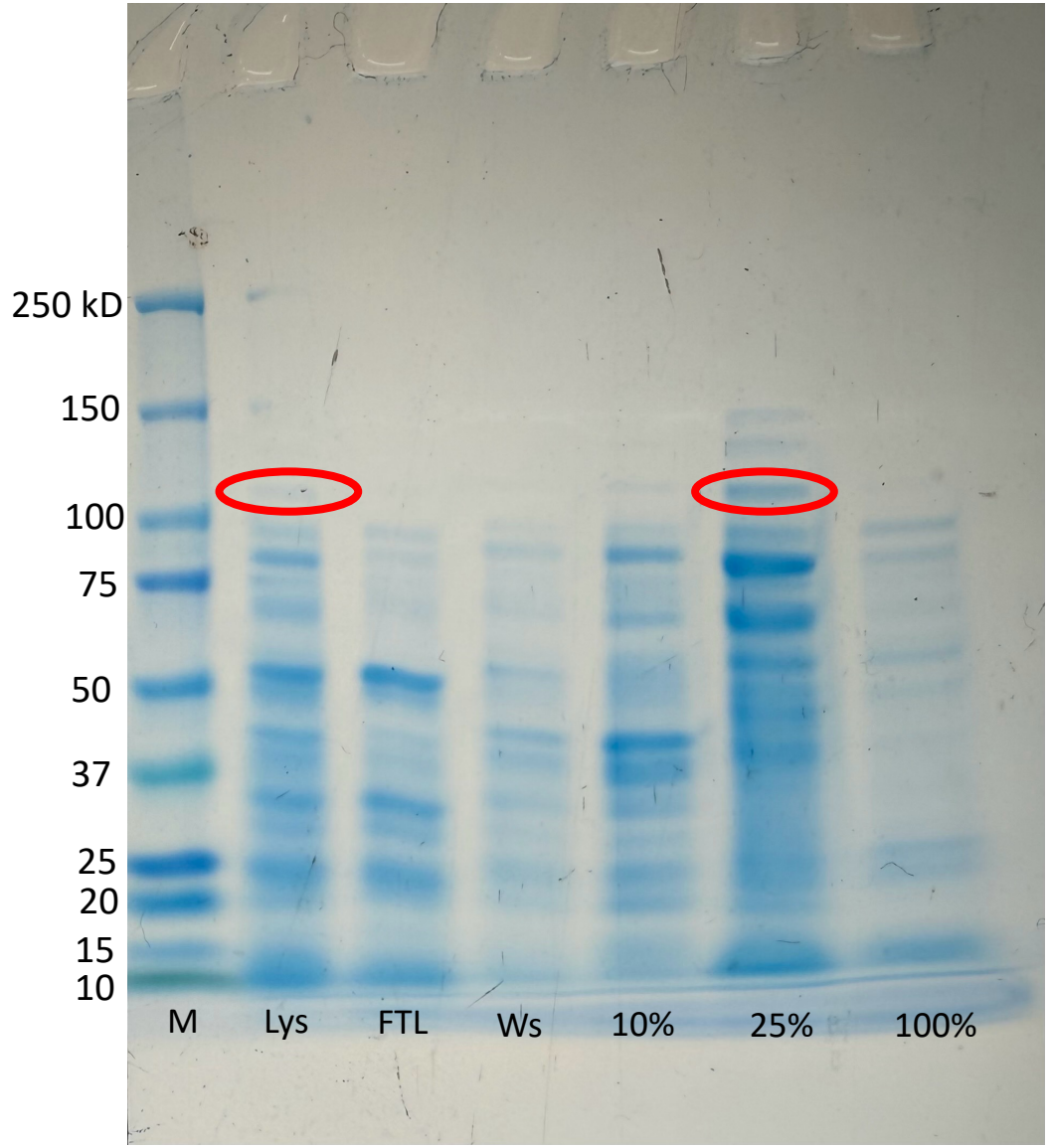
Ws: washing step

10%: eluate 10%

25%: eluate 25%

100%: eluate 100%

- We have >15 bands that are the most visible
- **Conclusion:** by AEXC we reduced the number of proteins in our sample to 15 (starting with 3000)

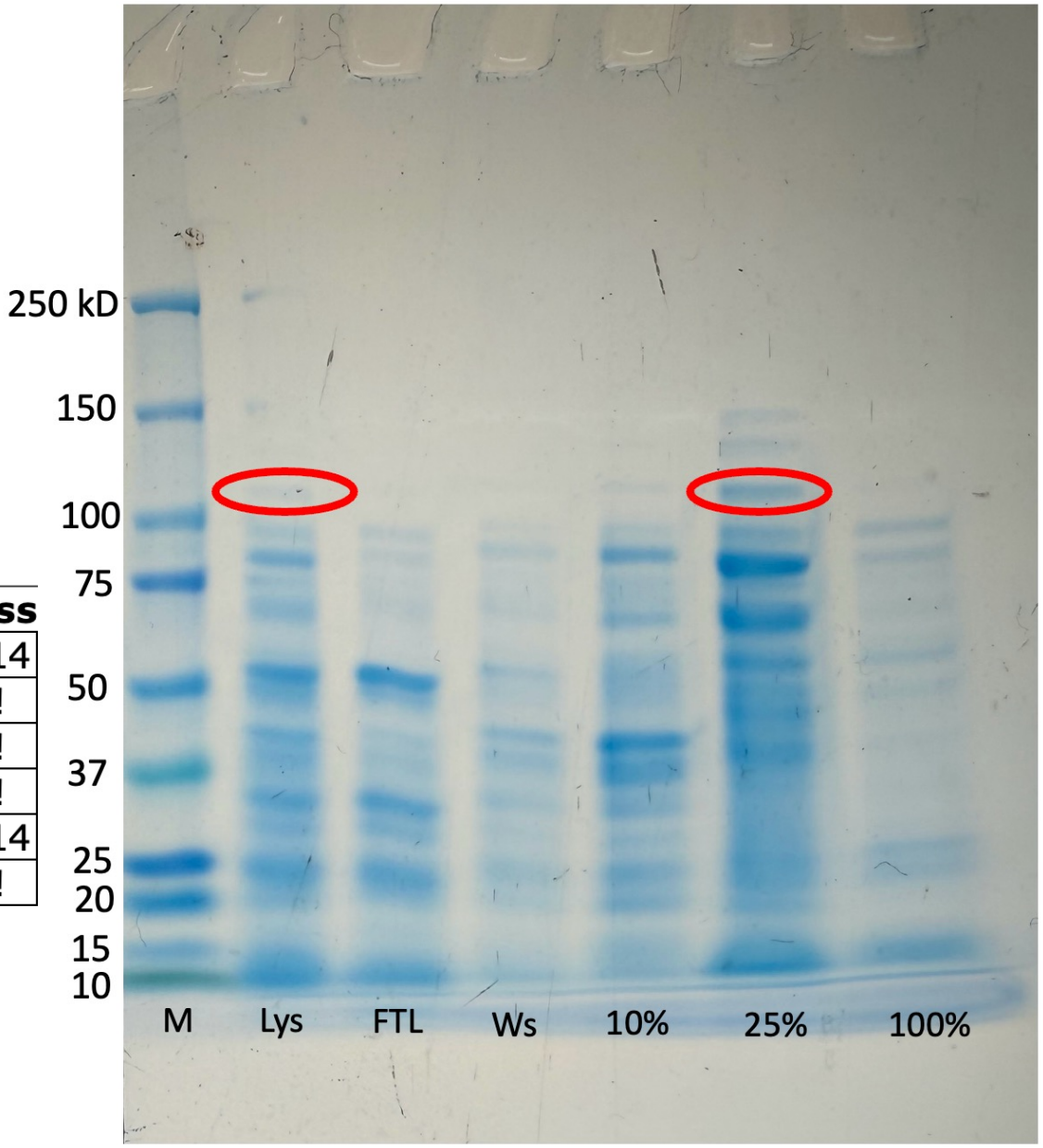


SDS - PAGE

- Identification of the lactase subunit

  in the gel with the marker

Sample	Migration rate (in cm)	molar Mass
Lysate deluted 1:10	1,7	114
Flow through of the loading	x	#VÆRDI!
Washing step	x	#VÆRDI!
Eluate 10%	x	#VÆRDI!
Eluate 25%	1,7	114
Eluate 100%	x	#VÆRDI!



Evaluation of the SDS – PAGE

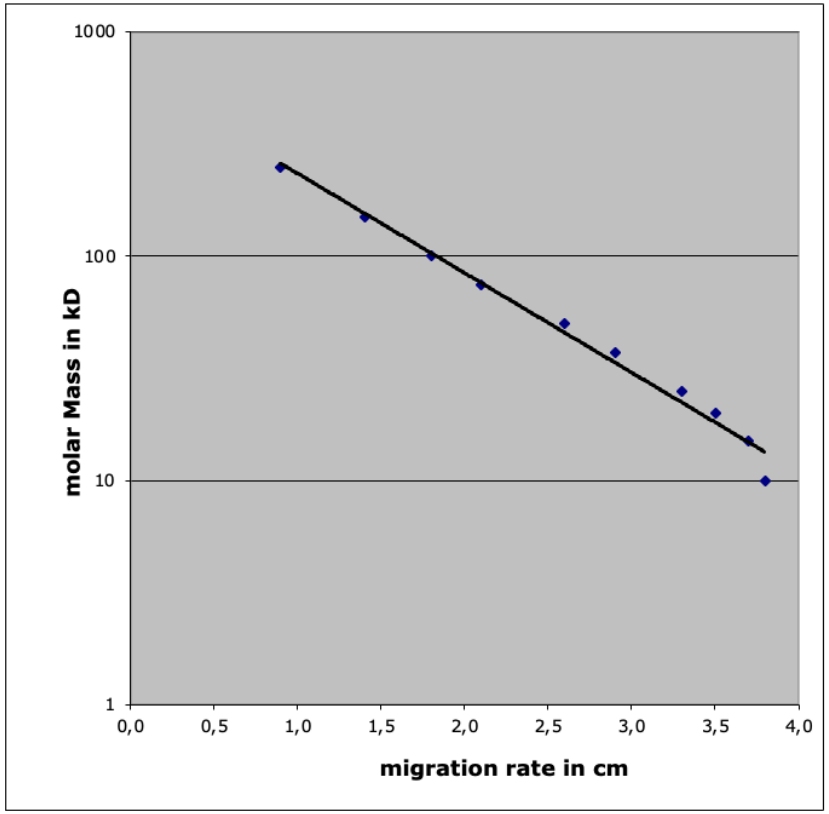
- Identification of molecular weight of lactase subunit with your calibration line

- **Migration rate:** 1,7 cm
- **Molecular weight:** 114 kDa

Precision Plus Protein Standard

migration rate (in cm	molar Mass
0,9	250
1,4	150
1,8	100
2,1	75
2,6	50
2,9	37
3,3	25
3,5	20
3,7	15
3,8	10

migration rate (in cm	molar Mass
1,7	114



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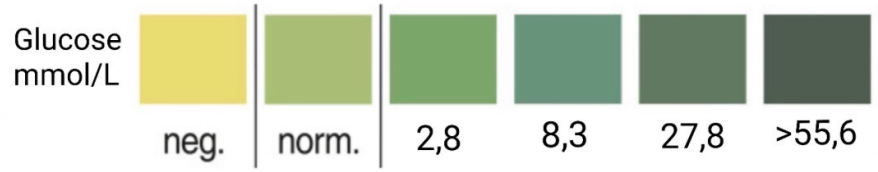
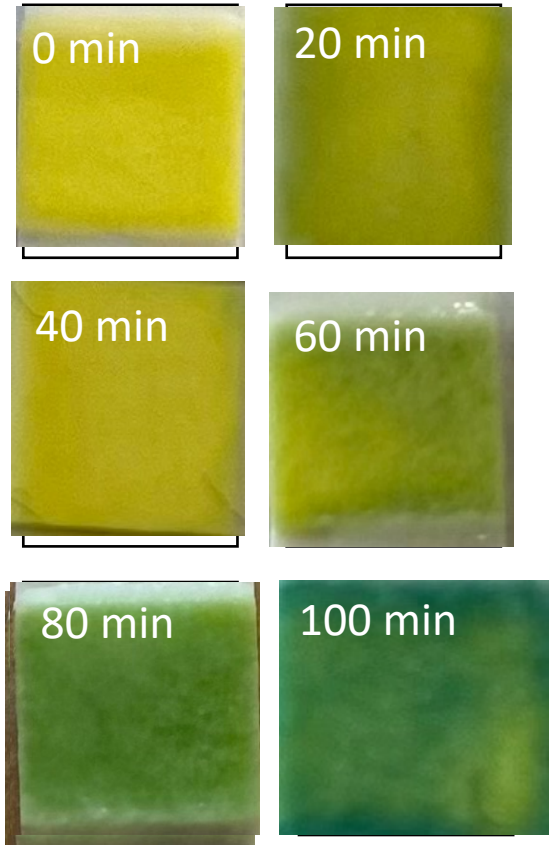
RAW MILK TEST



- **Observation:** colour of the stripe after 0/20/40/60/80/100 min got more green after longer time

- **Evaluation:** value of glucose after 100 min - ~28 mmol/L

- **Conclusion:** our isolated lactase could change raw milk containing lactose into lactose-free raw milk



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EVALUATION

1. TASK: Compare your results of AEXC according to the highest lactase activity and the highest amount of lactase in SDS – PAGE.

What can we conclude from the TASK 1?

According to both tests, the highest amount of lactase was in eluate 25%

2. TASK: sample, that would be suitable for downstream process and further purification of lactase – 25% eluate.

Thank you for your attention!