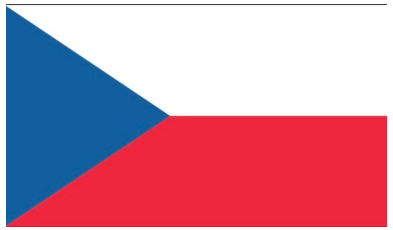
Summary of Results from Group 12 Lactase Downstream Processing

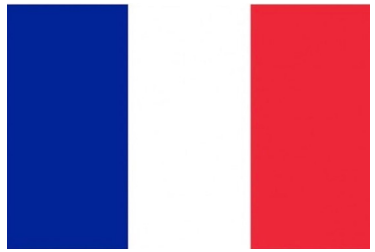
Evreux 23. – 27.03.2026

Group 12 + presentation of group members

Klára Koběrská
Czech Republic



Lou Bossut
France



Ayse-Naz Büyükbalbak
German

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:** Yellow
- **Evaluation:** β(1→4) bond was broken down
- **Conclusion:** lactase is present.

→ We can start AEXC.

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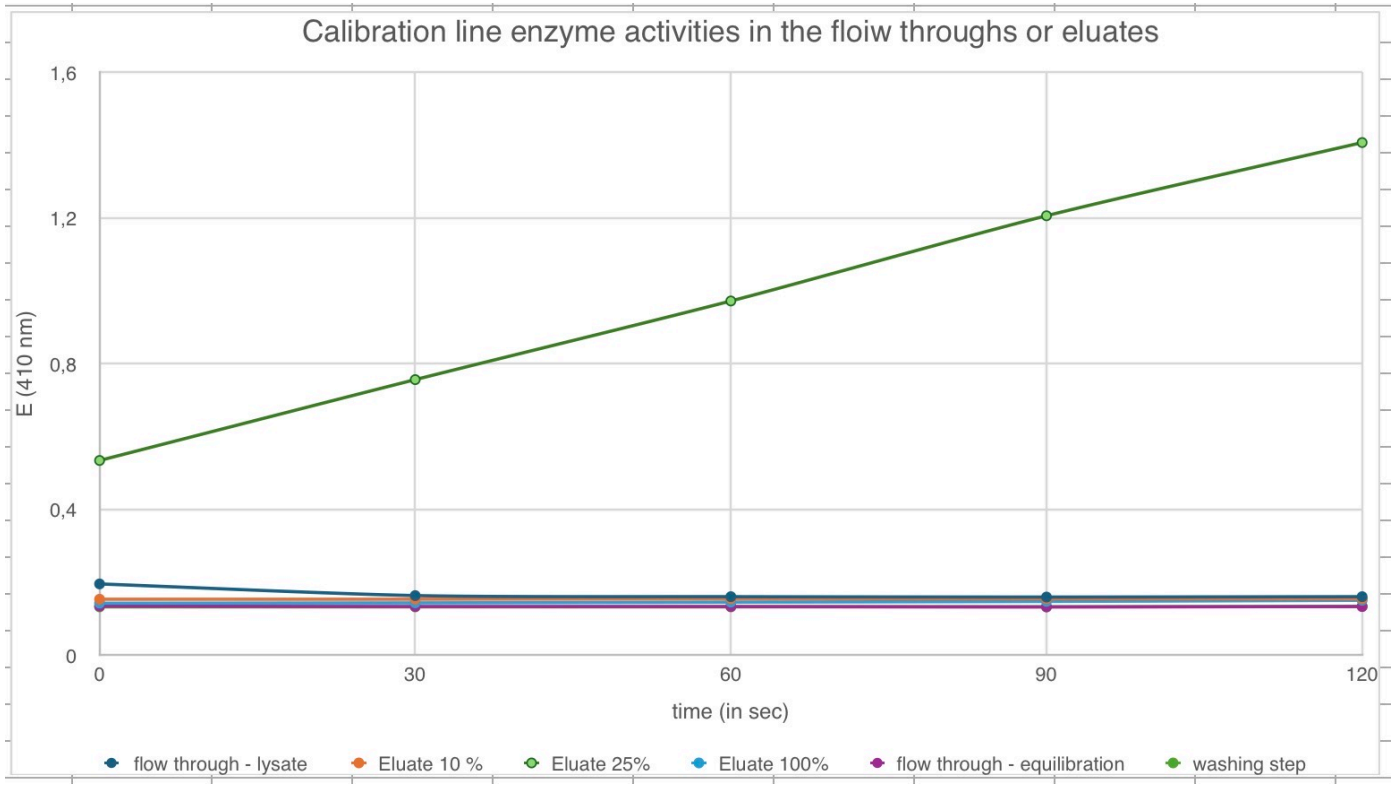
ANION EXCHANGE CHROMATOGRAPHY

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

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



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5. EVALUATION

SDS - PAGE

MARKER

S1: lysate (dilution factor 10)

S2: loading flow through

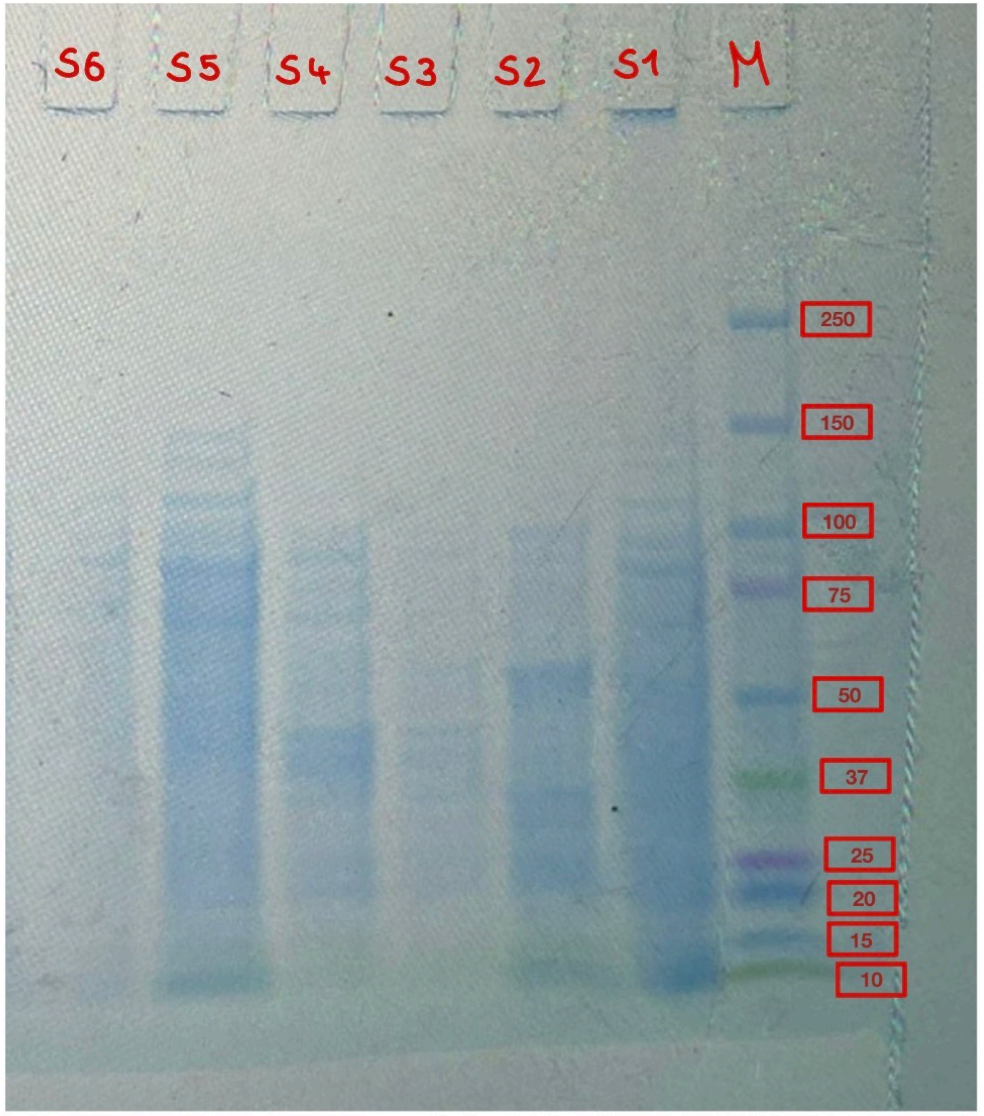
S3: washing step

S4: eluate 10%

S5: eluate 25%

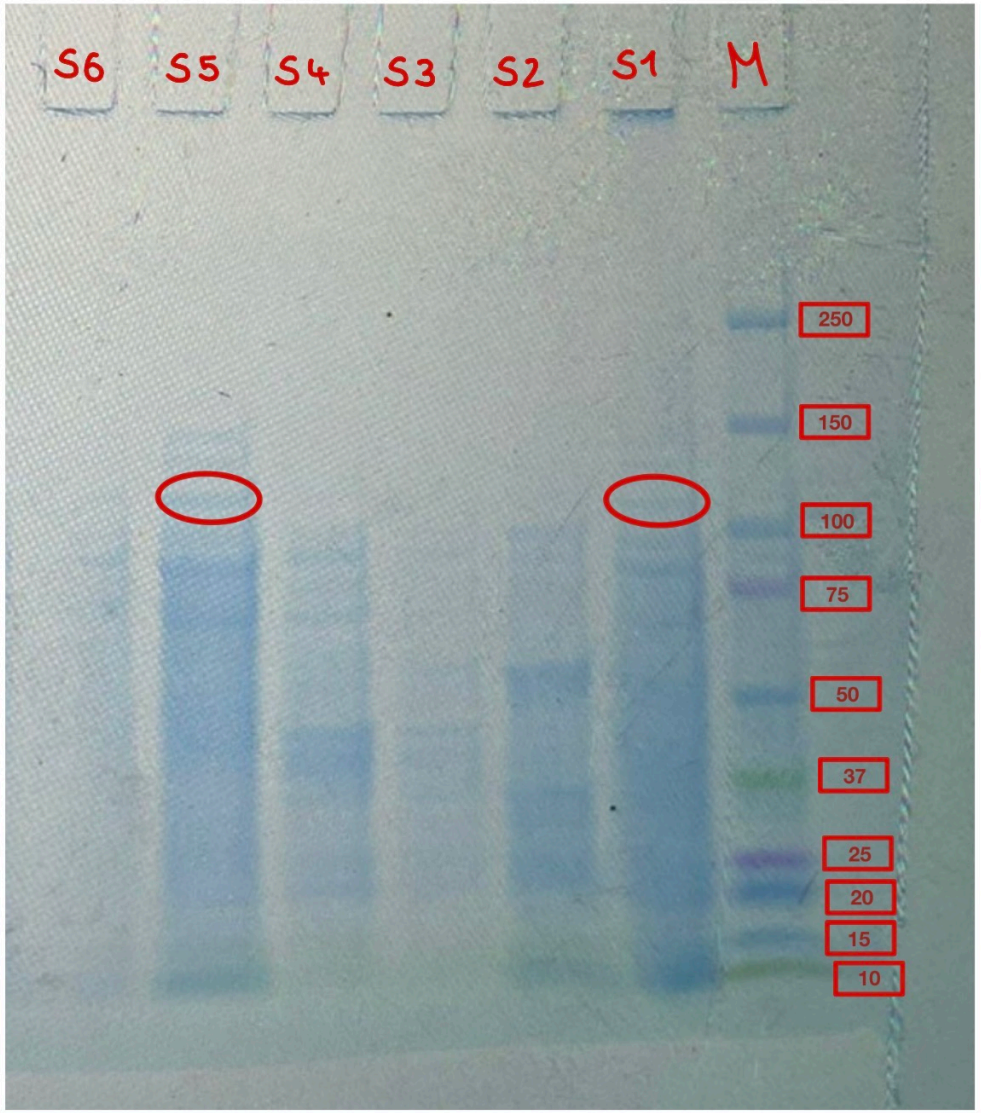
S6: eluate 100%

- **Conclusion:** by AEXC we reduced the number of proteins in our sample to an uncountable amount of proteins



SDS - PAGE

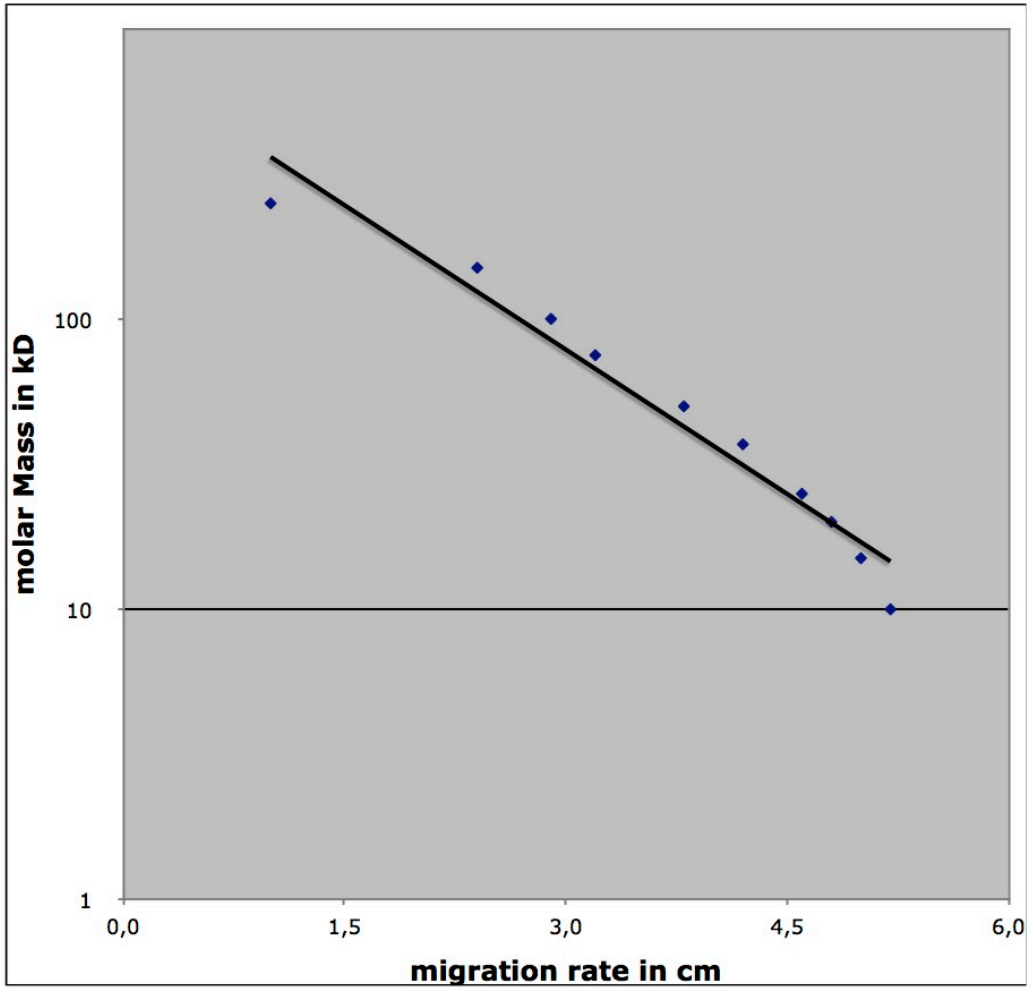
- We identification the lactase subunit (106kDa) in our gel using the marke



Evaluation of the SDS – PAGE

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

- **Migration rate: 2,6**cm
- **Molecular weight: 106** kDa



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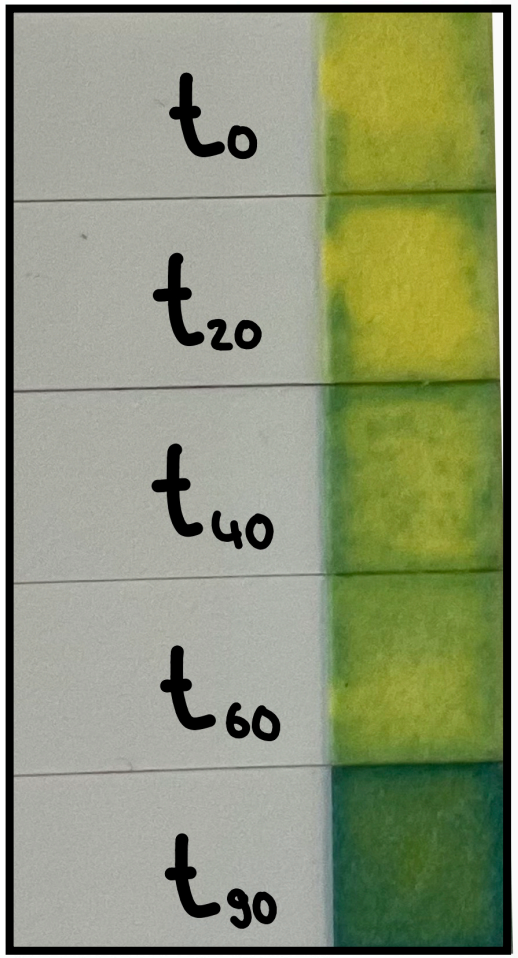
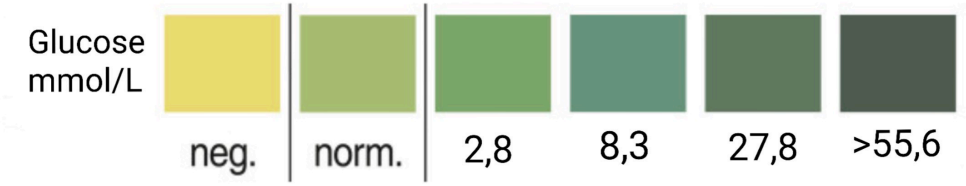
5. EVALUATION

RAW MILK TEST

- **Observation:** In the beginning the colour barely change. After 90 mins and some heat the sample turned green

- **Conclusion:** to create lactose free milk we could have to change the condition so the enzyme work better.





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EVALUATION

1. TASK: AEXC and SDS – PAGE showed the same results.

25% eluat had the highest amount of lactase

2. TASK: size exclusion chromatography

Thank you for your attention!