PROTEIN PURIFICATION His-tagged proteins

1. Prepare buffer W_{His} and D_{His}

W_{His} pH 7,5 (HCl) 50 mM Tris

300 mM NaCl

10 mM Imidazol

D_{His} pH 8 50 mM Tris- HCl

150 mM NaCl

buffer E_{His}

buffer W_{His} 150 mM Imidazol

wash the column with MQ and see if it is dripping properly.

Prepare the scaffold for the column.

Prepare Eppis for (Lysate, Flowthrough, Wash 1, Wash 2, Eluate 1-X (depending on the elution volume)

Load column material onto the column (about 2 ml)

Wash with EtOH

Wash with MQ

Wash with Buffer

sample of Lysate (20 µL for SDS gel)

Load Lysate

take flowthrough sample

wash with 2 ml Washbuffer (W1)
was with 20 ml Washbuffer (W2) (may be optional?)
prepare elution buffer

elute and collect all of it (E1-E7)

-> prepare all the SDS samples!

Samples for SDS-Gel:
L, D, W1

20 µL sample

10 µL SDS
20 µL MQ

W2 & E1-EX

40 µL sample

10 µL SDS

prepare the dialysis tube

fill the eluate into the tube and dialyse over night at 4°C

Next morning: Column with 10000kDa is stored on MQ.

Discard water, wash with water, then with dialysis buffer.

Load protein onto the column and spin at 4000 rpm for 5min, 4° C. Invert the tube, then spin again. Repeat until its only 2ml, measure concentration of protein, repeat until desired concentration. Take out the sample with yellow pipet, be careful NOT to touch the membrane!

Wash column with water and store on water.

SAMPLE PREPARATION FOR SDS PAGE

after addition of SDS sample buffer, cook samples at 95°C for 5-10 min.