His-Tag protein purification

The amino acid sequence of the polyhistidine tag is a sequence of at least six histidines whose gene sequence N-terminal is cloned after the start methionine codon or C-terminal before the stop codon into the open reading frame of a gene. The result is a fusion protein with a polyhistidine tag. Occasionally, an interface for a protease or an intein is inserted between protein and polyhistidine tag to enable cleavage of the tag after protein purification.1

Materials

Buffer A His-Tag (pH8.0: 50mM NaP; 500mM NaCl; 10mM Imidazole)
Buffer B His-Tag (pH8.0: 50mM NaP, 500mM NaCl, 350mM Imidazole)
Lysozyme
DNAse
Benzamidine
PMSF
MilliQ Water
Dialysis Buffer (DIA)

Instruments:

Sonifier
Centrifuge
Liquid pump
ÄKTApurifier
Glass flask
Measuring cylinder
Stir bar
Dialysis Equipment

Procedure

Lysis
- Add 3-5 times as much mL buffer A His-tag as mg pellet.
- Add a spatula tip lysozyme
- Add DNAse: 1/10000 of lysate volume
- Add benzamidine: 5mM final concentration
- Add PMSF: 0. 5mM final concentration

Sonification:
- Amplitude 50% (small peak); 30% (large peak)
- 5:00 min
- 0,5 Pulse on
- 2,0 Pulse off

Remove lysate -> take sample "Lysis Sample"
Centrifuge lysate 17k 50min 4°C
supernatant in glass flask -> take sample "supernatant sample”
resuspend pallet -> take sample "pellet sample”

Equilibrate His column (at 2 mL/min)

2-3x volume of column wash with MilliQ water
2-3x volume of column wash with buffer A His-tag
circulate 45min lysate through column -> take sample "flow through

ÄKTA

Connect buffer A and B His-Tag at ÄKTA
Start PumpWash
Connecting the column to ÄKTA
Run buffer A through column (until UV value has reached basal line)
Add 10% buffer B (wait until UV value reaches basal line)
Add 20% buffer B (wait until UV value reaches basal line)
Add 100% buffer B (reduce fraction size)
Removing fractions
Start PumpWash

Nanodrop measurement of fractions

Determination of the concentration via the specific extinction coefficient (https://web.expasy.org/protparam/)

Dialysis of samples

Add 1L dialysis buffer (DIA) to the measuring cylinder.
Moisten dialysisbag 4-7kDa with DIA buffer.
Attach knots and brackets and collected fractions as follows
Allow to stand overnight at 4 °C while stirring.

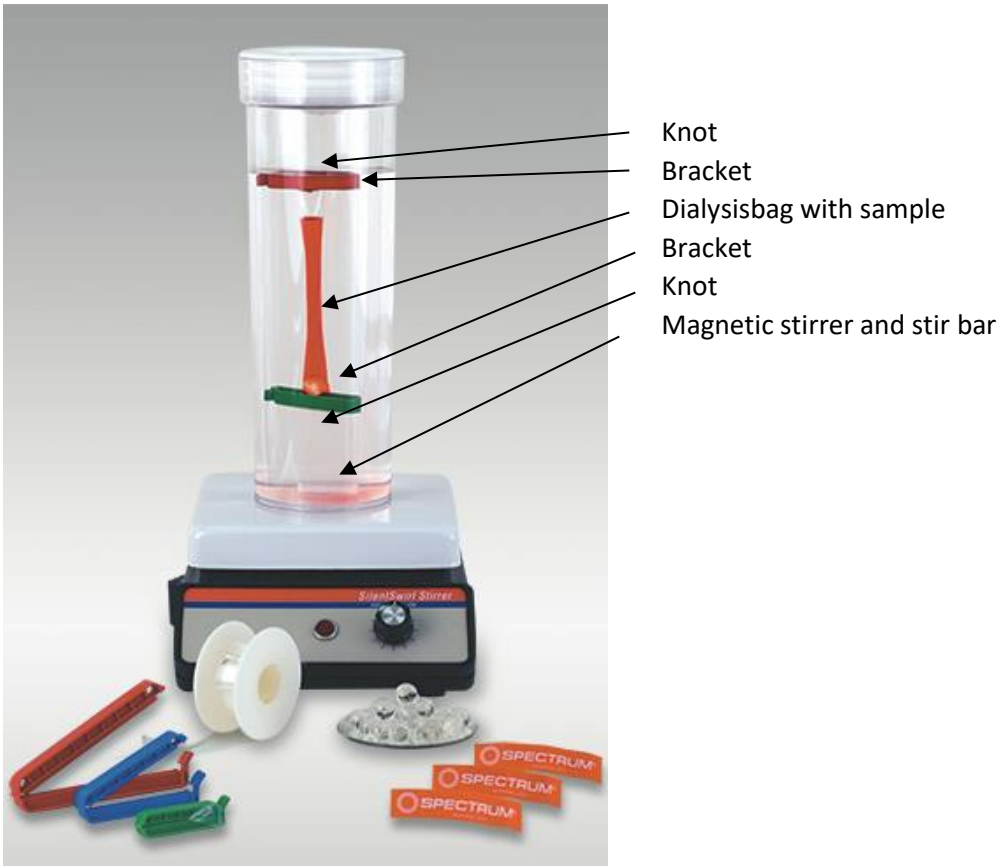

Figure 1: Dialysis of samples

New concentration determination with the Nanodrop
Freeze samples with liquid nitrogen and store at -80°C (add glycerol, if necessary)

Trouble shooting

The column should be thoroughly and long washed in the Äkta.
Since the affinity of the tagged protein is unknown, it should first be checked at which percentage of buffer B the protein is eluted.
The dialysis bag must be in a moving liquid to wash the buffer B out.
Fast working is important in this experiment in order to prevent additional denaturation of the proteins.
The work should take place from the lysis at 4°C.

References

[1] K. J. Petty: Metal-chelate affinity chromatography. In: Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.].

[2] Purification protocol provided by AG Binz

Figures:

[1]: http://de.spectrumlabs.com/dialysis/biotech.html