Strep-Tag protein purification

The Strep-Tag II is a protein tag that enables the purification and detection of recombinant proteins by affinity chromatography. It is a synthetic peptide which consists of 8 amino acids and can be expressed N- or C-terminal with a fusion protein. Strep-tag II has a strong affinity for strep-tactin, which is used for the purification of fusion proteins via affinity chromatography columns.1

Materials

Buffer A Strep-Tag (pH: 7. 4; 20mM NaP; 280mM NaCl; 6mM KCl)
Buffer B Strep-Tag (pH: 7. 4; 20mM NaP; 280mM NaCl; 6mM KCl; 2. 5mM Desthiobiotin)
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 Strep-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 Strep column (at 2 mL/min)

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

ÄKTA

Connect buffer A and B Strep-tag at ÄKTA
Start PumpWash
Connecting the column to ÄKTA
Run buffer A through column (until UV value has reached 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.

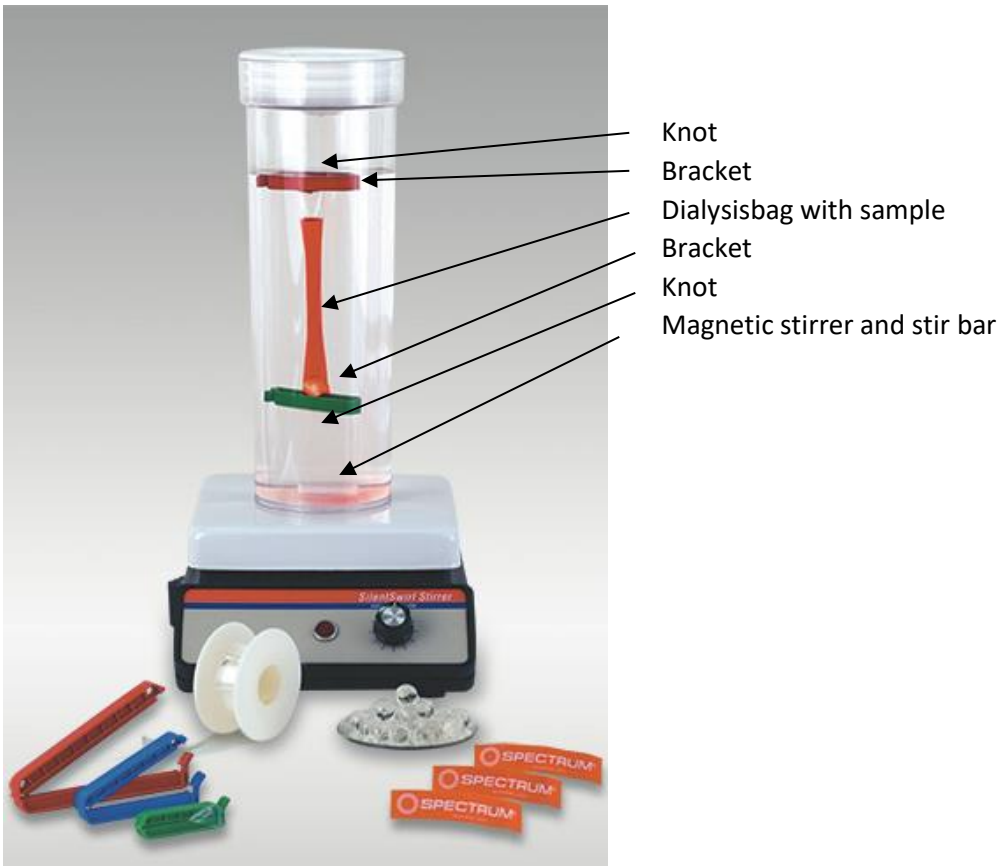

Figure 1: Dialysis of samples

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.
The dialysis bag must be in a moving liquid to wash the buffer B out.
Fast working is important in this process to prevent additional denaturation of the proteins.
The work should take place from the lysis at 4°C.

References

[1] A Skerra: Das Strep-tag als molekulares Werkzeug zur Hochdurchsatz-Proteinreinigung in der Proteomforschung. In: BIOspektrum. 2, 2003, S. 189-192.

[2] Purification protocol provided by AG Binz

Figures:

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