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Protein Purification using Strep-tag

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1 Protein Purification using Strep-tag

1.1 Buffer Preparation

Estimated bench time: 45 minutes
Estimated total time: 45 minutes
Purpose: To wash, elute and regenerate the column

1.1.1 Materials

- Trizma base (or Tris-HCl, but then use base to set the pH)
- NaCl
- Bottle (glass)
- Balance
- pH-indicator
- Acid
- Desthiobiotin (stored at 4°C)
- Regeneration Buffer stock (10X, stored at 4°C)
    - Work concentration: 100 mM Tris-HCl pH 8.0, 150 mM NaCl,1 mM EDTA, 1 mM HABA (hydroxyl-azophenylbenzoic acid)
- H_2O
- Graduated cylinder

1.1.2 Setup & Protocol

- Label the bottles and weight the right amount of each compound, following the table below.

Compound	Buffer Wash (200 ml)	Buffer Elution (100 ml)	Buffer NaOH stock (30 ml)
Trizma (100 mM)	2.42 g	1.21 g	-
NaCl (150 mM)	1.753 g	876.6 mg	-
Desthiobiotin (2.5 mM)	-	53.57 mg	-
pH	8.0*	8.0	-
NaOH	-	-	120 mg
MilliQ	-	-	30 ml

***optional: also make a wash buffer with pH 10.5 to better remove the regeneration buffer

- The Wash Buffer and NaOH stock can be stored at room temperature, the Elution Buffer at 4°C should only be made at the moment or only shortly stored at 4°C .

1.2 Preparing Column

Estimated bench time: 5 minutes
Estimated total time: 15 minutes
Purpose: Fill the column with the Strep-Tactin resin and let it sedimentate.

1.2.1 Materials

- Column
- Cap (bottom)
- Lid (above)
- Filter
- Lab stand
- Lab clamps
- Strep-Tactin resin (50%)
- (tweezer)
- Pipette Boy
- Pipette

1.2.2 Setup & Protocol

- Place the filter in the column (it can be helpful to use a tweezer).
- Clamp the column and make sure it stands vertical in the lab stand.
- Place the cap on the column
- Shake the resin and pipette 4 ml of the resin into the column for a 2 ml column. Other sizes can also be used, depending on the amount of protein that needs to be purified. Each column can be loaded with 0.5 till 10 times the column volume (for a Twin-Strep-tag, the maximum amount is 100 times the column volume).

1.3 Harvesting Proteins

Estimated bench time: 2 hours
Estimated total time: 3 hours
Purpose: Extract the cells form the culture media and break down the cells to dissolve the proteins, which can later be purified.

1.3.1 Materials

- Centrifuge
- Centrifuge tube
- Centrifuge bottles
- Lids
- Balance
- Bugbuster
- Benzonase Nuclease (enzyme 25U/µl)
- Agarose gel electrophoresis system

- DNA ladder
- Pipettes and tips
- Prepared samples
- Solidified agarose gel

1.3.2 Setup & Protocol

- Precool the centrifuge, 5 minutes at 2500 rpm. (Sorvall Evolution Centrifuge SLC-3000, Thermo Scientific)
- Weight the bottles when they are empty, so that you can later on determine the weight of the cell pellet.
- Harvest the cells by centrifuging at 4°C, 8000 rpm for 10 minutes. (Sorvall Evolution Centrifuge SLC-3000, Thermo Scientific)
- Keep the cell pellet and discard the supernatant.
    - Optional, snap freeze the cell pellet and store in -80°C and continue later.
- Weight the bottles and calculate the weight of the cell pellet.
- Use 5 mL of bugbuster per gram cell pellet
- Add 1 µl Benzoase Nuclease per 1 ml BugBuster
- Centrifuge the cell lysate at 4°C, 20000 rpm for 20 minutes. (Sorvall Evolution Centrifuge SA300, Thermo Scientific)
- Store the protein solution on ice.

1.4 Column Loading

Estimated bench time: 1 hour
Estimated total time: 1 hour
Purpose: Purifying the protein by loading it on a Strep-Tactin column

1.4.1 Materials

- Colum (Strep-tactin)
- Lab stand
- Buffers:
    - Wash
    - Elution
    - NaOH (Dilute the stock to 10 mM)
- Pipette
- Eppes
- Erlenmeyer

1.4.2 Setup & Protocol

- As no filter has been placed on top of the resin, pipette buffer or protein very gently onto the column in each step. It is recommended to pipette to the column wall instead of directly on the resin. To make a column of 2 mL for example you need 4 mL.
- Equilibrate the column using 2 column volumes of wash buffer(~8 mL).

- Load the supernatant on the column (maximum is 10 times the column volume but depends on the concentration). Collect a drop of the flow-through for SDS-PAGE analysis.
- Wash the column using 3-5 column volumes of wash buffer (~10 mL). (Make sure no fluorescently colored sample is released from column). Collect a drop of the flow-through for SDS-PAGE analysis
- Elute the protein from the column by the addition of 3 column volumes of elution buffer (~6 mL). Collect the elution, as it contains the purified protein. Make sure all protein is eluted.
- Wash using 2 column volumes (~4 mL) 10mM NaOH.
- Wash using 8 column volumes (~8mL) of wash buffer
- Store the column at 4° C overlaid with 2 column volumes (~4 mL) of wash buffer.

1.5 Buffer Exchange

Estimated bench time: 1 hour
Estimated total time: 1 hour
Purpose: changing the buffer (wash) of the protein to the desired buffer

1.5.1 Materials

- Amicon Ultra tube with filter
  - Filter sizes: 3 kDa, 10 kDa, 30 kDa, 50 kDa, or 100 kDa
  - Volumes: 0.5 ml, 2 ml, 4 ml, or 15 ml
- Centrifuge
- Protein Buffer (values depend on the protein and the purpose)
  - Salt: 150 mM NaCl
  - Ph: 7.2
  - Tris: 25 mM
- Pipette
- Pipette tips

1.5.2 Setup & Protocol

- Load the protein sample on the filter.
- Place the filter tube in the centrifuge (minispin or table centrifuge, depending on the filter tube volume), balance well.
- Set the spin-time and rpm. A good first spin time is 4-5 minutes. Afterwards, you can test how much of the buffer went through the filter and how much longer you want to continue. It is also good to mix the protein solution, as it can form a layer at the surface of the filters. The necessary rpm depends on the tube, for 15 ml an rpm of 4000 is sufficient, while for the tube of 0.5 ml a rpm of 13500 is better.
- Repeat the loading of the protein buffer till the original protein sample buffer is sufficient diluted. A dilution of at least 30x is recommended.
- After the buffer exchange, you can measure the protein concentration with nanodrop and preform a QTOF to see if the protein had the right size.