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Protein Purification using His-tag

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1 Protein Purification using His-tag

Estimated bench time: 2 hours
Estimated total time: 3 hours, depending on the amount of samples
Purpose: To wash, elute and regenerate the column

1.1 Materials

- Protein samples
- Charge Buffer (80 mL)
- Wash Buffer (300 mL)
- Elution Buffer (150 mL)
- Strip Buffer (150 mL)
- Columns
- Ni-NTA HisBand resin
- H_2O
- Filter for on top of the resin
- Eppendorf tubes
- TCEP
- Pipette boy

1.2 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.

1.2.1 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

1.2.2 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 Benzonase 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.

1.3 Column Loading

- Prepare the buffers needed for protein purification:

Charge buffer

- 100 mM $NiSO_4$

T14-3-3cΔC wash buffer B

- 50 mM Tris
- 300 mM NaCl
- 20 mM imidazole
- 2 mM TCEP
- pH 8.0

T14-3-3cΔC wash buffer A

- T14-3-3cΔC wash buffer B
- 0.1% Triton-X-100
- pH 8.0

T14-3-3cΔC elution buffer

- 50 mM Trizma
- 300 mM NaCl
- 250 mM imidazole
- 2 mM TCEP
- pH 8.0

Assay buffer

- 10 mM HEPES
- 150 mM NaCl
- pH 7.4

strip buffer:

- 0.5 mM NaCl
- 100 mM EDTA
- 20 mM tris hCl
- pH 7.9

- Load a column with Ni-NTA HisBind resin (5-10 mg protein per mL resin). For example load 6 mL of resin for a 3 mL column.
- Add a filter on top of the resin. Make sure that no air gets trapped in the resin or between filter and resin.
- Equilibrate the column with 6 column volumes H_2O (~18 mL)
- Add 3 column volumes of Charge Buffer (~9 mL). Collect the waste flow-through separately as this is heavy metal waste (has to be thrown away in specific jerry can in fume hood)
- Add 3 column volumes of Wash Buffer B (~9 mL) to remove unbound nickel. Collect the waste flow-through again separately (has to be discarded in halogen rich waste).
- Load the protein samples (supernatant from Bugbuster cell lysis) onto the column. Collect a drop of the flow-through for SDS-PAGE analysis
- Add 3 column volumes of wash buffer A and 3 column volumes of wash buffer B (~18 ml total). Collect a drop of the flow-through for SDS-PAGE analysis
- Elute the protein by adding 3 column volumes of elution buffer (~9 mL). Collect the coloured elution sample in 0.5-1 mL fractions in Eppendorf tubes. Store a drop of the flow-through for SDS-PAGE analysis
- Keep the protein elution fractions on ice in a closed bucket
- Strip the Ni^{2+} off the column using 2 column volumes of strip buffer (~12 mL). Once more it is necessary to use a separate waste disposal container.
- Wash with 5 column volumes of H2O
- To preserve the column it is necessary to add 2 column volumes (~6 mL) of 20% ethanol.
- Store the column in the fridge at 4 °C

1.4 Buffer Exchange

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

1.4.1 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

1.4.2 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.