Immobilised Metal Affinity Chromatography (IMAC)

Introduction

IMAC is a technique that uses a nickel-charged resin to bind his-tagged protein for later elution. This process removes unwanted impurities and proteins which either do not bind to the resin, elute at a lower imadazole concentration, or remain bound when the target protein is eluted.

Materials

- PD-10 empty disposable columns for gravity flow purification (cytiva)
  - Empty column
  - Column top cap
  - Column bottom cap
  - Polyethylene filter discs
- Chelating sepharose fastflow (cytiva)
- 20% ethanol
- MilliQ water
- Charge Buffer (100mM nickel sulphate)
- 1X Binding Buffer (10mM imadazole)
- 60 Wash Buffer (60mM imadazole)
- Elution Buffer (500mM imadazole)
- 3x Falcon tubes
- 7x 2mL Eppendorf tubes
- Clamp and clamp stand
- Additional receptacle for catching waste fluid
- Relevant buffer for buffer exchange (varies)
- Liquid nitrogen

Procedure

Packing the columns

1. Place the polyethylene filter disc into the empty column. Ensure that the rough side of the disc is facing upwards and the bottom is flush with the base of the column.

2. Attach the column to the clamp stand and have it hover vertically above the waste receptacle.

3. Place the bottom cap onto the column.

4. Ensuring that it is resuspended beforehand, pour 5mL chelating sepharose into the column.

5. Add 2.5 column volumes (12.5mL) of MilliQ water, keeping the column as full as possible, and remove the bottom cap. This will wash out the ethanol holding the resin whilst also applying pressure, packing the resin. Ensure that there is a small reservoir above the surface of the resin at all times to prevent the resin from drying out.

Charging the resin

6. Add 2.5 column volumes (12.5mL) of Charge Buffer to prime the resin for his-tag chelation.

7. Add 5 column volumes (25mL) of MilliQ water to wash out the excess Charge Buffer.

8. If storing the column, add 2.5 column volumes (12.5mL) of 20% ethanol, add both caps, and place vertically in the fridge (4c).

Purifying the protein

9. If taking the column from storage, add 2.5 column volumes (12.5mL) of MilliQ water and allow it to run through into the waste receptacle. This is done to wash out all ethanol.

10. Add 2.5 column volumes (12.5mL) of 1X Binding Buffer to equilibrate the resin.

11. Replacing the recaptacle with a Falcon labelled "Flow-Through", add all supernatant sample.

12. Replacing the previous Falcon with one labelled "Binding Buffer Wash", add 2.5 column volumes (12.5mL) of 1X Binding Buffer.

13. Replacing the previous Falcon with one labelled "60 Wash", add 2.5 column volumes (12.5mL) of 60 Wash Buffer.

14. Replacing the previous Falcon with a 2mL Eppendorf tube labelled "Elution 1", add 14mL of Elution Buffer.

    There should be 7 2mL Eppendorf tubes in total, labelled "Elution 1" through to "Elution 7". Every time a 2mL Eppendorf tube becomes full, switch the capture receptacle to the next tube in line. It is important to be careful during this step, as it is easy to come into contact with the Elution Buffer during tube-switches.

15. You should now have 3 Falcon tubes and 7 Eppendorf tubes worth of samples. Buffer exchange into the relevant solution should be performed as soon as possible prior to storage to prevent precipitation of the purified protein (rendering it unusable in several assays, such as Bradford Assay).

16. Flash freeze the buffer-exchanged samples in liquid nitrogen and place immediately into the -80c freezer.

Storing the column

17. Add 2.5 column volumes (12,5mL) of MilliIQ water to wash out the excess Elution Buffer.

18. Add 2,5 column volumes (12.5mL) of 20% ethanol, add both caps, and place vertically in the fridge (4c). Do not reuse the column for any protein other than the one it has already been used for.