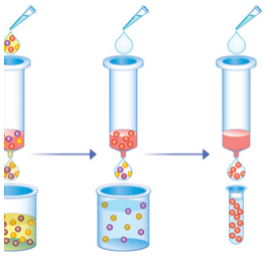
Oct 24, 2020

His-tag purification



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Andreea S[1]

[1]University of Groningen

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Protocol status: Other
The protocol is developed based on literature and has not been tested yet.

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Abstract

His tag purification uses the technique of immobilised metal affinity chromatography. In this technique, transition metal ions are immobilized on a resin matrix using a chelating agent such as iminodiacetic acid. It has been studied that among amino acids constituting proteins, histidine is strongly involved in the coordinate bond with metal ions. Therefore, if a number of histidines are added to the end of the protein by genetic engineering, the affinity of the protein for the metal ion is remarkably increased and the basic idea is that purification can be easily carried out. When a protein having a His tag is brought into contact with a carrier on which a metal ion such as nickel is immobilized, the histidine residue chelates the metal ion and binds to the carrier. Since other proteins do not bind to the carrier, they can be washed off with a buffer. Thereafter, it is possible to recover the protein having the His tag with high purity.

Separate proteins from soil matrix

1 Collect soil samples of 5 g

2 Extract total proteins using NoviPure Soil Protein Kit or other comercially available kits for total protein soil extraction

His-tag separation of NLP

1h

3 Wash the Ni^{2+}-sepharose column material with 12 CVs of MQ and 4 CVs of Column Wash Buffer ([M] 10 millimolar (mM) imidazole, KPi [M] 50 millimolar (mM) pH 7 , NaCl [M] 200 millimolar (mM)).

> Note
>
> Use ±0.5 ml of Ni^{2+}-sepharose column material per 10 mg of total protein.

4 Apply the sample, add imidazole (10mM final concentration) and the washed Ni^{2+}-sepharose column material. Nutate in at 4 °C for 01:00:00 1h

5 Pour column, collect flow through to apply on SDS gel.

6 Wash column with 20 CVs of Wash Buffer ([M] 50 millimolar (mM) imidazole, KPi [M] 50 millimolar (mM) pH 7 , NaCl [M] 200 millimolar (mM)).

7 Elute protein with Elution Buffer ([M] 500 millimolar (mM) imidazole, Kpi [M] 50 millimolar (mM) pH 7 , NaCl [M] 200 millimolar (mM)) in 200 µL fractions. Check elution fractions Absorbance by NanoDrop.

8 Run an SDS gel to check purification:

- Soil suspension & Flow through: dilute 15x, apply 5 µL
- Wash: dilute 1.25x, apply 10 µL
- Elution fractions: dilute to ±0.2 mg/ml, apply 5 µL