

ÄKTA Pure Purification System

Aim of the experiment

ÄKTA Pure is a commercial purification system for fast and effective purification of peptides and proteins using automated liquid chromatography.

Materials

Tris-HCl (pH 8.0)

NaCl

Imidazole

IPTG

Lysozyme

Benzamidine

PMSF

20% Ethanol

Medium for culture

Ni-NTA-column

SDS-PAGE

10 kDa MWCO centrifugal filters

2 x 2.5L sterile Shaking flasks

2ml micro-centrifuge tubes

Preparation

1. Prepare Wash Buffer according to the following table.

Concentration (mM)	Reagents
50	Tris-HCl (pH 8.0)
500	NaCl
25	Imidazole

2. Prepare Elution Buffer according to the following table.

Concentration (mM)	Reagents
50	Tris-HCl (pH 8.0)
500	NaCl
250	Imidazole

3. Prepare Storage Buffer according to the following table.

Concentration (mM)	Reagents
20	Tris-HCl (pH 8.0)

Procedure

1. Prepare 10 ml overnight culture.
2. Inoculate 2 shaking flasks (500 ml) each with 5 ml overnight culture.
3. Induce culture with 1 mM IPTG.
4. Overnight the expression of the protein at 20°C.
5. Harvest cells by centrifugation at 4,000 g.
6. Resuspend cells in 40 ml Wash Buffer.
7. Add 1 mM Lysozyme, 1 mM benzamidine and 1 mM PMSF.
8. Lyse the cells by sonication at 50% amplitude, with 12 cycles of 30 seconds pulse + 90 seconds pause.
9. Centrifuge the solution for 30 minutes at 7190 g.
10. Transfer supernatant to 2ml micro-centrifuge tubes.
11. Centrifuge at 21,000 g for 10 minutes, then pool the supernatant.
12. Equilibrate Ni-NTA-column, with 10 col. vol. water, 10 col. vol. Elution Buffer and 10 col. vol. Wash Buffer.
13. Load column with cell lysate.
14. Wash column with 10 col. vol Wash Buffer.
15. Elute the protein with 10 col. vol Elution Buffer; (linear gradient between 25 mM and 250 mM of imidazole was used), then collect 1 ml fractions.
16. Wash again with 10 col. vol. water and 10 col. vol. 20% ethanol.
17. Store the Ni-NTA-column.

17. Analyze the collected fractions on a SDS-PAGE and measure the protein concentration by photometric measurement.
18. Pool the fractions which contain the desired protein.
19. Carry out buffer exchange by 10 kDa MWCO centrifugal filters with 3 washing steps to store the proteins.
20. Reanalyze the purity of the samples on a SDS PAGE and measure protein concentration by photometric measurement.
21. Store the proteins at -20°C.