H-NS Protein purification

pI of H-NS = 5.1 – should require an anion exchange column!

Grow 1L culture to OD650 of 0.600.

Induce with 1 mM IPTG for two hours.

Collect cells and resuspended in 10ml buffer A [20 mM Tris-HCl (pH 7.2), 1 mM EDTA, ~~5 mM β-mercaptoethanol~~, 10% glycerol], containing 100 mM NaCl and 1 mg/ml PMSF.

Sonicate and clear lysate by centrifugation at ~~37 000~~ 13,000 r.p.m. for ~~2.5~~ 1 h at 4°C.

The supernatant was directly loaded onto a ~~P11~~ Heparin column pre-equilibrated with the same buffer.

A linear ~~NH4Cl~~ NaCl gradient up to 1 M was applied and H-NS eluted around ~~800~~ 500 mM ~~NH4Cl~~ NaCl.

The buffer with NH_4Cl was replaced with buffer A, containing 100 mM NaCl by dialysis overnight.

Dilute fractions to 50ml with 20mM Tris 1mM EDTA

~~Subsequently the dialysate was loaded onto a pre-equilibrated Heparin column.~~ sample was loaded on QFF column*

H-NS eluted around 550 mM NaCl. H-NS containing fractions were dialysed against a buffer solution containing 20 mM Tris-HCl (pH 7.2), 300 mM KCl and 10% glycerol.

All buffers contained 100 µg/ml PMSF (Sigma) and 2 mM benzamidine (Sigma) to prevent proteolytic cleavage of the H-NS protein. The purity of the H-NS protein was verified on an SDS-PAGE gel and the protein concentration was determined with the Bio-Rad protein assay (Bio-Rad).

*(Doesn't work well. SFF column better)

Gel ①

1	Marker	
2	F2	
3	F33	] H-NS ~ 17ml
4	F 34	
5	F 35	
6	F 36	
7	F 37	
8	F 38	
9	F 39	
10	F 40	
11	F 41	
12	F 42	
13	F 43	
14	F 44	
15	F 45	

Gel ②

1	Marker	
2	F20	
3	22	
4	24	] H-NS but dirty
5	25	
6	26	
7	30	
8	31	
9	32	
10	33	
11	34	
12	35	
13	36	
14	37	
15	38	

⇒ Try again using SFF column.
↳ Works much better