Purification secreted holo-Fet3pFlag protein

1) Media

CM-URA w/2X glucose buffered 50mM Tris-acetate pH 6.5
supplemented with 100µM CuSO₄

2) Cells

4L AFT1%secFet3pFlag (W303 background)

3) Inoculation

one half of a 100mm plate streaked in a grid pattern (culture on plate should not be more than 3 days old)
scraped with sterile cell scraper (Costar)
1/2 scraped plate/1L of media/2L Fernbach flask

4) Growth

Overnight 30°C
air shaker 250rpm
2L Fernbach flask

5) Harvest

Put flasks on ice
-Pack cells 5,000xg for 15min., decant supernatant into 2L bottle, keep on ice
For every 2L of supernatant add 8.37g Bis-Tris(Sigma), 100µl 200mM BCS(bathocuproinedisulfonic acid, disodium salt hydrate),
plus 20ml of 1M NaOH(brings pH to approximately 7.2)
Prefilter using .45µ SFCA(surfactant free cellulose acetate) membrane(Nalgene)
Filter again using .2µ membrane
Apply cold supernatant to column packed with Source 30Q beads(Pharmacia), equilibrated in 20mM Bis-Tris pH 7.2
(column run in 4°C cold room)
(all buffers .2µ filtered)
-Wash column with at least 4L(equal to or greater than the original load volume)
Elute using 20mM Bis-Tris pH 7.2 w/300mMNaCl
SecFet3p starts to come off the column just after reaching void volume
O.D. of fractions taken at A₂₈₀nm to determine peaks of protein, pPD assay(p-Phenylenediamine dihydrochloride)
and Western analysis(probed anti-Flag) done on fractions were used to determine fraction pools
Silver stain analysis was used to determine purity of pools
Purified protein was washed using 20mM Bis-Tris pH 7.2 until salt concentration was negligible; using Centriprep50
and then the protein was concentrated using a Centricon50(Amicon)
Purified protein was N-linked deglycosylated with EndoHf(NEB) according to manufactures directions
Purified secFet3pFlag protein was separated from EndoHf using a small G75 Sephadex column
This purified protein is what was injected into mice