Purification of dnEBNA-1/Softag1 from E. coli BL21 LysS (p3134)

1. Inoculate 2ml of 5ml o/n culture of either p3133 (empty vector pET11a) or p3134 (dnEBNA-1/Softag1) in E. coli BL21 LysS per 0.5L LB + ampicillin (grow two 0.5L cultures of each)
2. Incubate ~2hrs @37C 250rpm until OD_{600} = 0.4-0.6
3. Induce with 5ml 100mM IPTG per 0.5L culture
4. Incubate 2-3hrs @37C 250rpm
5. Spin down 2 flasks of each (1L total) in 500ml GSA centrifuge bottles 5000rpm, 4C, 10min
6. Decant supernatant, freeze pellet @-20C
7. Resuspend pellet in TED+0.15M NaCl (2-5ml per gram wet weight of pellet; for bacterial pellet from 1L I used 6ml on 02/2004)
8. Add 200x lysozyme and 100x proteasome inhibitors to 1x final concentration
9. Transfer to disposable, sterile tube and incubate on ice 30min (omitted on 02/2004)
10. Sonicate setting 10, 15-30sec bursts, 6 rounds, incubate on ice ~2min between rounds
11. Transfer to microfuge tubes; Centrifuge 4C, 10k rpm 30min
12. Transfer supernatant to fresh tube.
13. Column Preparation (**mAb NT73-Sepharose4B resin**)
    a. Resuspend matrix as 50% slurry and load closed column
    b. Wash twice with 5 bed volumes of TED+0.15M NaCl
    c. Open column and drain to form compact 100% slurry
14. Load sample onto column. *Note: all flow rates should be 0.5ml/min*
15. Collect Flow Through (FT), pass FT over column again. Save 50ul of supernatant as **FT**.
16. Wash with 25ml TE+0.15M NaCl. Collect this wash and save 50ul as **Wash 1**.
17. Wash with 15ml TE+0.5M NaCl, collect in 5ml fractions. Save 50ul of each as **Wash 2.1, Wash 2.2, Wash 2.3**.
18. Elute dnEBNA-1/Softag1 from column with 5-10ml TE+0.7M NaCl+30% propylene glycol, collect in 1ml fractions. Add 5ul of 0.02M DTT to each 1ml fraction. Save 50ul of each fraction as **Elution 1, 2, 3, 4, 5 (6, 7, 8, 9, 10)**.
19. Run 2 identical SDS-PAGE gels with all **bold** samples above, protein size marker, and concentration standards (BSA 0.1ug, 1ug, 10ug) or protein positive for Softag1 and/or EBNA-1
    a. Run one gel as a Coomassie Blue stain for bulk protein levels
    b. Run one gel as Western for identification of Softag1 and/or EBNA-1

<u>TED+0.15M NaCl</u>
10mM Tris pH 7.4
1mM EDTA (pH 8)
150mM NaCl
0.1mM DTT

<u>TE+0.5M NaCl</u>
10mM Tris pH 7.4
1mM EDTA (pH 8)
500mM NaCl
10% glycerol (v/v)

<u>TE+0.7M NaCl+30% propylene glycol</u>
10mM Tris pH 7.4
1mM EDTA (pH 8)
750mM NaCl
30% propylene glycol (v/v)