VINCULIN full length, expression and purification

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Method Article

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Abstract

Introduction

Expression and purification of vinculin from bacterial culture.

Procedure

Starter Culture and Induction 1. Pick a single colony \(or from glycerol stock) into 25 ml TB + salts + 0.4% glucose + antibiotic 2. Grow until mid-log phase \(OD600 ~ 0.4-0.5), 37 °C 3. Store at 4 °C O/N 4. Inoculate 2x 500 ml TB + salts + 0.4% glucose + antibiotic with 10 ml @ of starter culture 5. Grow to OD600 = 0.4-0.6 6. Induce with 1mM IPTG, 37 °C, 3 hours 7. Pellet cells and resuspend in 20 ml of lysis buffer \(300 mM NaCl, 10 mM Imidazole, 20 mM Tris pH 8) 8. Quick freeze in Liquid Nitrogen. Store at -80 °C until needed **Purification** 1. Thaw Pellet\(s) in room temperature water 2. Add 2.5 ml 10 mg/ml lysozyme \(10 mg/ml lysozyme in H_2O stored at –20 °C) and 0.25 ml 100 mM PMSF \(dissolve 100mM PMSF in EtOH and store at –20 °C) add βME to 5mM final to each pellet. 3. Sonicate, large probe, 3-5 minutes, 1 second on 2 seconds off, on ice 4. Spin 18 K, 1 HR in SS-34 5. Wash HiTrap Chelating HP 5ml column \(Amersham 17-0409-03) with 10 ml 6 M GuHCl \(skip if a new column) 6. Wash column with 50 ml dH_2O 7. Load column with 20 ml of 0.1 M $NiCl_2$ 8. Wash column with 50 ml dH_2O 9. Wash column with 25 ml \(5 column volumes) 10 mM Imadazole wash buffer \(10 mM Imadazole, 500 mM NaCl, 20 mM Tris pH 8) 10. Load sample containing 10 mM Imadazole, 300 mM NaCl, 20 mM TRIS pH 8, collect flow through. Check on gel. 11. Wash column with 25 ml 10 mM Imadazole buffer \(10 mM Imadazole, 500 mM NaCl, 20 mM Tris pH 8). Collect wash to check on gel. 12. Wash column with 25 ml 25 mM Imadazole buffer \(25 mM Imadazole, 500 mM NaCl, 20 mM Tris pH 8). Collect wash to check on gel. 13. Wash column with 25 ml 75 mM Imadazole buffer \(75 mM Imadazole, 500 mM NaCl, 20 mM Tris pH 8). Collect wash to check on gel. 14. Elute sample with 12 ml 250 mM Imadazole buffer \(250 mM Imadazole, 500 mM NaCl, 20 mM Tris pH 8). Check on gel 15. Strip column with 10 ml EDTA strip buffer \(50 mM EDTA, 500 mM NaCl, 20 mM Tris pH 8). Collect strip to check on gel. 16. Rinse column with 50 ml dH_2O, store at 4 °C 17. Dialyze eluted, 12 ml sample against Buffer A \(150 mM NaCl, 20 mM Tris pH 8, 5 mM EDTA, 5 mM βME) overnight at 4 °C. 18. Dialyze 3 or more hours against Buffer B \(150 mM NaCl, 20 mM Tris pH 8, 5 mM βME). 19. Add 4 aliquots Thrombin \(5 Units), 4 °C Overnight 20. Add Imidazole to a final concentration of 250mM, NaCl to a final of 500mM. 21. Repeat Nickel column Steps 5-18 \(cleaved protein should be in the FT and Wash 1). 22. Add another 2 aliquots of thrombin \(2.5 U) to elution, RT overnight. 23. Repeat Step 21. 24. Concentrate to appropriate concentration and run over S-200 column.