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VINCULIN HEAD Domain 1 (Vd1 and Vd1 Y100E mutant): expression and purification

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Method Article

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Abstract

Introduction

Protocol describes expression and purification of vinculin head domain 1.

Reagents

Vector - pET15b-VD1 Molecular Weight = 30.8 KD Ext = 10870 to get μM Ext = 0.355 to get μg/μl **Binding Buffer** 5mM Imidazole 500mM NaCl 20mM Tris-HCl pH 7.9 **Wash Buffer** 30mM Imidazole 500mM NaCl pH 8.0 20mM Tris-HCl pH 7.9 **Elution Buffer** 75mM Imidazole 500mM NaCl pH 8.0 20mM Tris-HCl pH 7.9 **Dialysis Buffer 1** 25mM Tris-HCl pH 8.0 150mM NaCl 5mM EDTA 5mM βME **Dialysis Buffer 2** 25mM Tris-HCl 150mM NaCl 5mM βME **ITC Buffer** 20mM Tris-HCl 150mM NaCl

Procedure

Amplification 1) 100ml o/n LB/amp culture per 800ml culture the next day, 37 °C. 2) Seed 800ml LB/amp culture with 100ml o/n culture. Grow at 37 °C. until OD600 = 0.6-1.2. Induce with 1mM IPTG. Grow 3hr. Harvest. 3) Spin 8krpm, 15min, 4 °C. 4) Resuspend in His Binding Buffer. Snap freeze. **Purification** 1) Lyse 2x800ml cell pellets with homogenizer. 2) Spin 16krpm, 30min, 4 °C. 3) Load sup on to 3ml equilibrated Ni-NTA column. 4) Wash with 50ml binding buffer. 5) Wash with 10 column volumes wash buffer. 6) Elute with elution buffer. 7) Dialyze protein into dialysis buffer 1. 8) Concentrate VD1 to 20-30mg/ml. 9) Dialyze protein into dialysis buffer 2 or end use buffer. 10) SDS PAGE on purification. 11) Dialyze protein into appropriate experimental buffer.