Tensin SH2-PTB (ScPc) expression and purification

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Method Article

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Abstract

Introduction

Methodology describing the expression and purification of the SH2-PTB domain portion of tensin.

Reagents

pGEX4T1-GST-ScPc 30.3KD w/o GST-tag pI = 8.5 Ext = 0.559 **Cleavage Buffer** 50mM Tris-HCl pH7.0 \(at 25°C) 150mM NaCl 1mM EDTA 1mM DTT **Dialysis Buffer** 10mM Tris-HCl pH 7.4 50mM NaCl 0.5mM EGTA 1mM DTT

Procedure

Expression 1) 100ml o/n culture in LB/amp. 2) Seed 1L TB/amp with 50ml o/n culture. 3) Grow at 37°C until OD600 = 0.6-1.2. 4) Induce with 1mM IPTG. 5) Grow for 3hr, 37°C. 6) Harvest \(8krpm, 4°C, 10min). Resuspend pellet in 20ml PBS. 7) LN_2 snap freeze. **Purification** 1) To 1L cell pellet add 1 crushed PI tablet. 2) Lyse cells with 0.2mg/ml lysozyme, 15min rocking or until very thick. 3) Add 10mM $MgCl_2$. 4) Clear DNA with 40µg/ml DNaseI, 15min rocking or until runny. 5) Add 0.1% TX-100. Rock 15min. 6) Spin, 16krpm, 30min, 4°C. 7) Wash 2ml Glutathione Sepharose 4B with 50ml PBS. 8) Rock beads with supernatant for 1hr, 4°C. 9) Load onto a column and collect flow through. 10) Wash beads with 50ml PBS. 11) Wash beads with 50ml Cleavage Buffer. 12) Cleave protein off the beads with 100µl PreScission Protease in 2ml Cleavage Buffer. Rock overnight \(16hr, 4°C). 13) Elute the next day with Cleavage Buffer. 14) Dialize into Dialysis buffer. Do protein concentration. Snap Freeze. 15) SDS PAGE. 16) Can further purify on Superdex 75 \(10/300) to get rid of upper MW band \(do 500µl at a time).

Troubleshooting

Chill cleavage buffer to 4°C before using.