Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Tensin-PTBc expression and purification

Robert Liddington
The Burnham Institute
Andrey Bobkov
The Burnham Institute

Method Article

Keywords: tensin, PTB

Posted Date: February 5th, 2009

DOI: https://doi.org/10.1038/nprot.2009.41

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Introduction

Protocol describes expression and purificaton of PTB domain from tensin in bacterial culture.

Reagents

pET15b-His-Tensin-PTBc \(aa1653-1786) MW 15.5KD Ext Coef 0.484 \(or 7090 to get µM) **Binding Buffer \(High Salt)** 5mM Imidazole 500mM NaCl 20mM Tris-HCl pH 7.9 1mM DTT **Elution Buffer \(Low Salt)** 300mM Imidazole 100mM NaCl 20mM Tris-HCl pH 7.9 1mM DTT **Wash Buffer \(Low Salt)** 30mM Imidazole 100mM NaCl 20mM Tris-HCl pH 7.9 1mM DTT **Dialysis Buffer** 20mM Tris-HCl pH 7.9 100mM NaCl 1mM DTT

Procedure

Expression 1) 100ml o/n LB/amp culture 2) Next day seed 700ml LB/amp with 100ml o/n culture, 37°C. 3) Grow cells until OD600 = 0.6-1.2 4) Induce with 1mM IPTG. Grow for 3hr 37°C. 5) Harvest 8krpm, 4 °C, 10min. 6) Resuspend 800ml cell culture pellet in 20ml Ni-NTA binding buffer. LN_2 freeze. **Purification** 1) Thaw 1L cells. Add PI tablet w/o EDTA. Add 2mM PMSF. 2) Add 0.4mg/ml lysozyme. Rock 15-30min until thick. 3) Add 10mM $MgCl_2$. Add 0.4µg/ml DNaseI. Rock 15-30min until loose. 4) Add 0.1% Tx-100. Rock 15min. Save 10µl for SDS-PAGE. 5) Spin 16krpm, 4°C, 15min. Save some supernatant and pellet for gel. 6) Meanwhile, wash 2ml Ni-NTA beads with binding buffer. 7) Load supernatant onto beads. 8) Wash with 50ml binding buffer. 9) Wash with 50ml wash buffer low salt. 10) Elute with elution buffer low salt. 11) Dialize into dialysis buffer while digesting off His tag with biotinylated thrombin. Digest for 6hr RT. Capture biotinylated thrombin with streptavidin agarose. 12) Run SDS PAGE on purification. Determine if need to do further purification on S75 or S200. Do a quick protein concentration check. Concentrate if necessary.