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Expression and purification protocol of linear GST-4xUbiquitin

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ABSTRACT

This protocol details the expression and purification of linear GST-4x Ubiquitin.

ATTACHMENTS

246-481.docx

DOI

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PROTOCOL CITATION

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KEYWORDS

linear GST-4xUbiquitin, Protein purification, Protein expression

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OWNERSHIP HISTORY

Jun 05, 2021 Urmilas

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PROTOCOL INTEGER ID

50499

MATERIALS TEXT

General information:

Sequence Analyzer: ATP1A1_plasmid_donor_RD Sequencing

Result **addgene Catalog #171421**

A	B
Expression system	E.Coli BL21DE3
Medium	Luria Bertani
Plasmid origin	Addgene 171421
Backbone	pGEX5
Resistance	Amp
Insert	Mus Ubiquitin (NM_001313984.1)
Tags & cleavage sites	N-term GST no TEV cleavage site
Ext coeff	46060 M-1cm-1, MW 58.9 kDa

Lysis Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	300 mM
TCEP	1 mM
Protease Inhibitors (Roche)	

Wash Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	300 mM
TCEP	1 mM

Elution Buffer:

A	B
Hepes pH=7.5	50 mM
NaCl	300 mM
TCEP	1 mM

SEC Buffer:

A	B
Hepes pH=8	20 mM
NaCl	200 mM
TCEP	1 mM

Columns/Resin: - Glutathione Sepharose 4B
S200 (GE Helathcare)

Protein expression 4h 20m

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1 Transform the E.Coli BL21DE3 cells with a plasmid encoding for GST-tetraubiquitin and plate them on Amp plate.

2 4h

Carry out the protein expression in **1.5 L** LB meidum, induce with **100 Micromolar (µM)** IPTG (isopropyl- β-d-thiogalactopyranoside) to an OD_{600} of 0.8 and grow at **18 °C** **Overnight** .

3 20m

Harvest the cells by spinning at **4500 x g** for **00:20:00** at **4 °C** and stock at **-80 °C** until purification.

Protein purification 5h

4 Follow the GST batch purification by Size Exclusion Chromatography.

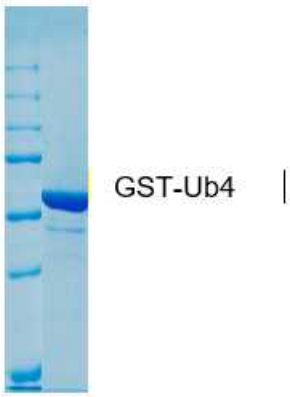
5 Resuspend the pellets in Lysis Buffer, sonicate for cell lysis and clear at **16000 rpm** at **4 °C** for **01:00:00** 1h

6 4h

Incubate the supernatant with Glutathione Sepharose 4B (GE Healthcare) at **4 °C** with gentle shaking for **04:00:00** , apply to a gravity flow column, and wash extensively with Wash Buffer.

7 Elute the protein of interest with Elution Buffer and then apply onto a Superdex 6 column (10/300 Increase) pre-equilibrated in SEC Buffer operating at **4 °C** .

8 Pool the peak fractions containing pure protein, store the snap-frozen in liquid nitrogen at **-80 °C** .



Yield: about 7.5 mg per liter culture

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