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Expression and purification protocol of GST-mCh-FYVE

Forked from Expression and purification protocol of GST-NDP52

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details the expression and purification of GST-mCherry-FYVE.

Attachments

DOCX

246-484.docx

1.4MB

Materials

General information:

	A	B
	Expression system	E.Coli BL21DE3
	Medium	Luria Bertani
	Plasmid origin	
	Backbone	pGST2
	Resistance	Amp
	Insert	
	Tags & cleavage sites	N-term GST
	Ext coeff	82320 M-1cm-1, MW 77.9 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	150 mM
	TCEP	1 mM

Elution Buffer:

	A	B
	Hepes pH=7.5	50 mM

	A	B
	NaCl	150 mM
	TCEP	1 mM
	Glutathione	50 mM

SEC Buffer:

	A	B
	Hepes pH=8	20 mM
	NaCl	150 mM
	TCEP	1 mM

Columns/Resin:

- Glutathione Sepharose 4B
- S6_10/300 Increase

Protein expression

20m

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

2 Carry out protein expression in 1 L medium, induce with [M] 200 micromolar (µM) IPTG (isopropyl- β-d-thiogalactopyranoside) to an OD_{600} of 0.8 and grow at 18 °C Overnight .

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

20m

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 Incubate the supernatant with Glutathione Sepharose 4B (GE Healthcare) at 4 °C with gentle shaking for 00:30:00 , apply to a gravity column, and wash extensively with Wash Buffer.

30m

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 at 4 °C .

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