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EXPRESSION AND PURIFICATION OF HUMAN NEMO (GST-GFP-NEMO)

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes how to express and purify human NEMO (IKK- γ) tagged N-terminally with GST and EGFP. The expression is performed with *E. coli* Rosetta pLysS cells. The protein is purified via a GST batch purification and gel filtration (SEC).

Materials

pGEX-GST-Thrombin-TEV-GFP-NEMO (Addgene ID: 199781)

E. coli Rosetta (DE3) pLysS cells

LB medium with antibiotics: 50 μ l/ml ampicillin and 34 μ l/ml chloramphenicol

IPTG (Isopropyl- β -D-thiogalactopyranosid)

Thrombin (Serva, #36402.01)

Columns/Resin:

Glutathione Sepharose 4B (Cytiva, #17075605)

Superose 6 increase 10/300 column (Cytiva, #17517201)



Before start

Lysis Buffer:

50 mM HEPES pH 7.5

300 mM NaCl

2 mM MgCl₂

Freshly added: 2 mM β -Mercaptoethanol, Roche Protease Inhibitor (Merck, #5056489001), and DNase I (Sigma, #DN25-1G)

Wash Buffer 1:

50 mM HEPES pH 7.5

300 mM NaCl

Freshly added: 1 mM DTT

Wash Buffer 2:

50 mM HEPES pH 7.5

700 mM NaCl

Freshly added: 1 mM DTT

SEC Buffer:

25 mM HEPES pH 7.5

150 mM NaCl

Freshly added: 1 mM DTT



Expression

16h 15m

- 1 Grow *E. coli* Rosetta (DE3) pLysS cells in  2 L LB medium at  37 °C until achieving an OD_{600 nm} of 0.4.
- 2 Reduce temperature to  18 °C and continue growth to OD_{600 nm} of 0.8.
- 3 Induce protein expression with  250 micromolar (μM) IPTG and continue growth for ~  16:00:00 at  18 °C 16h
- 4 Centrifuge the cells at 3000 rcf ( 4 °C ,  00:15:00) 15m
- 5 Aspirate media and resuspend cell pellet in ~ 10 ml of 1X PBS, centrifuge the cells at 3000 rcf ( 4 °C ,  00:15:00) and take off the supernatant. 15m
- 6 Flash freeze the pellet in liquid nitrogen and store at  -80 °C until use.

Purification

46m 30s

- 7 Thaw pellet and resuspend in Lysis Buffer with freshly added  2 millimolar (mM) β-Mercaptoethanol, Roche Protease Inhibitor and DNase I
- 8 Sonicate sample  00:00:30 at 65% power for 5 cycles (Bandelin Sonopuls) 1m 30s
- 8.1 Repeat sonication for a total of 3X
- 9 Centrifuge at 48000 rcf at  4 °C for  00:45:00 45m
- 9.1 During this step, equilibrate  3 mL Glutathione Sepharose 4B beads by washing the slurry with water and Wash Buffer 1 (~ 6 ml).



10 Filter supernatant after centrifugation through a  0.45 µm syringe filter

11 Incubate the sample on equilibrated beads on a tube roller at  4 °C for

4h

 04:00:00

12 Wash beads 5X with Wash Buffer 1

13 Wash beads 1X with Wash Buffer 2

Note

These wash steps remove nonspecific proteins

14 Wash beads 2X with Wash Buffer 1

Note

These washes remove high salt concentration

15 Cleave the resulting sample to produce GFP-NEMO by incubating with thrombin

1d

 Overnight at 

16 Centrifuge beads at 3000 rcf for  00:03:00 at 

3m

17 Collect supernatant and filter through  .45 µm syringe filter

18 Concentrate product to a final volume of  500 µL using a 10 kDa MWCO concentrator

19 The concentrated and filtered protein can be applied onto a Superose 6 increase column (10/300 Cytiva) pre-equilibrated with SEC buffer



Note

This step can remove contamination and degradation products

- 20 Fractions containing the purified proteins are pooled, concentrated, frozen in liquid nitrogen and stored at - 80°C

Note

Protein purity can be determined by SDS PAGE analysis and measurement of protein concentration