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

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

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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 µl/ml ampicillin and 34 µl/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)

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BEFORE START INSTRUCTIONS

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 [M] 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 [M] 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 4h

Incubate the sample on equilibrated beads on a tube roller at 4 °C for 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 1d

Cleave the resulting sample to produce GFP-NEMO by incubating with thrombin Overnight at 4 °C

16 3m

Centrifuge beads at 3000 rcf for 00:03:00 at 4 °C

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-equillibrated 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