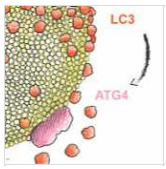

Jun 01, 2021

Expression and purification protocols of Homo sapiens GST-ATG4B recombinant protein

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ABSTRACT

This protocol describes expression and purification procedures for obtaining GST-tagged human recombinant Autophagy specific de-ubiquitinase ATG4B (AuTophaGy-related protein).

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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Feb 25, 2021 Dorotea Fracchiolla Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna (AT)

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GUIDELINES

General information

Insert: Homo sapiens ATG4B, NM_013325.5; Expression system: E.Coli Rosetta pLyss; plasmid origin: Sascha Martens Lab, lab internal construct database number SMC1392; backbone: pGEX-4T1; plasmid resistance: Ampicillin; tags & cleavage sites: N-term GST, followed by Thrombin cleavage site, ATG4B ORF. Ext coeff: 65890 M^{-1} cm^{-1}, MW 44,3 kDa.

MATERIALS TEXT

Materials and Reagents

- Escherichia coli Rosetta pLyss cells
- Luria Bertani (LB) medium with antibiotics (final conc. 50µg/ml Ampicillin, 34µg/ml Chloramphenicol)
- IPTG (isopropyl-b-d-thiogalactopyranoside)
- 37°C shaker incubator
- sterile flasks/sterile pipettes
- tip sonicator
- Thrombin (SERVA)

Buffers

- Lysis Buffer: 50 mM Hepes pH=7.5, 300 mM NaCl, 2 mM ß-mercaptoethanol (or 1mM DTT, Dithiothreitol), 1mM $MgCl_2$, spatula tip DNAse (Sigma), Complete Protease Inhibitor Roche EDTA-free (1 tablet/50ml buffer);
- Wash Buffer 1, 3: 50 mM Hepes pH 7.5, 300 mM NaCl, 1 mM DTT (Dithiothreitol);
- Wash Buffer 2 (high salt): 50 mM Hepes pH 7.5, 500 mM NaCl, 1 mM DTT (Dithiothreitol);
- Gel Filtration Buffer: 20 mM Hepes pH=7.5, 200 mM NaCl (filtered and degassed) + 1 mM DTT (Dithiothreitol)

Note: all purification buffers are filtered and degassed. Reducing agents (β-mercaptoethanol and Dithiothreitol) are added after degassing step.

Columns:
- GSH beads stored in 20% Ethanol (Pierce or Glutathione Sepharose 4B);
- S75_16/60 (GE Healthcare).

Gels: 12% SDS-PAGE

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Protein expression

1 Transform **1 µl mini DNA plasmid SMC1392 (~100ng/µl)** into one aliquot (**50 µl**) of chemically competent E.coli Rosetta pLyss cells according to standard protocol.

2

Plate cells on Amp/Cam Ty (or LB) plate and grow **Overnight** at **37 °C** .

3 The following day, pick 2-3 colonies to inoculate a pre-culture of ~ **150 mL LB (Amp/Cam)** and grow **Overnight** at **37 °C** .

4 In the morning, use 4 x **25 mL pre-culture** to inoculate 4 x **1 L LB (Amp/Cam)** .

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5 Grow cultures **shaking** at **37 °C** until OD_{600} (Optical Density) = 0.4 and then switch to **18 °C** .

6 Induce cultures **when cooled down** and OD_{600} =0.9 is reached with **1 Milimolar (mM) IPTG** and carry protein expression **Overnight** at **18 °C** .

7 

The following day, harvest cells by spinning at **4000 rpm, 4°C, 00:10:00** in a Sorvall RC6+ centrifuge (Thermo Scientific).

8 Resuspend cell pellets in ~ **25 mL ice cold lysis buffer** / **1 L cell culture** , flash freeze in liquid nitrogen and store at **-80 °C** .

Protein purification 11h

9 

Wash **10 mL slurry GSH beads** stored in 20% Ethanol (Pierce or Glutathione Sepharose 4B) in milliQ water and finally equilibrate in cold WB1. Perform spinning at **4000 rpm, 4°C, 00:03:00 , brake=2** in a 5810R centrifuge (Eppendorf).

10 Thaw frozen resuspended cell pellets in **Room temperature** water bath and move **On ice** as soon as thawed.

11 Sonicate cells 2x30s 50% cycle, 70% Power at **4 °C** using a tip sonicator.

11.1 Sonicate cells for **00:00:30** 50% cycle, 70% Power. (1/2) 30s

11.2 Sonicate cells for **00:00:30** 50% cycle, 70% Power. (2/2) 30s

12 Collect Lys sample for gel loading.

13 

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Clear lysate in a Beckmann Ultracentrifuge for **25000 rpm, 4°C, 00:45:00 , Ti45 rotor** .

14 Collect supernatant sample.

15 

Incubate supernatant with **5 mL GSH beads pre-equilibrated in WB1** .

16 Perform binding at **4 °C** for **01:00:00** , rolling. 1h

17 

Wash beads 2x in WB1.

Each wash can be performed by gently inverting the tube a few times or by rolling the tube for 10 minutes at 4°C-> spin at 4000 rpm for 3 min at 4°C.

17.1 Wash beads in WB1. (1/2)

17.2 Wash beads in WB1. (2/2)

18 

Take "sup W1" sample. Take "beads WB1". Wash 2x in WB2.

19 

Take "sup W2" sample. Take "beads WB2". Wash 2x in WB3.

20 Take "sup WB3" sample. Take "beads WB3".

21 Check all fractions on a 12% SDS-gel stained with Coomassie Brilliant Blue.

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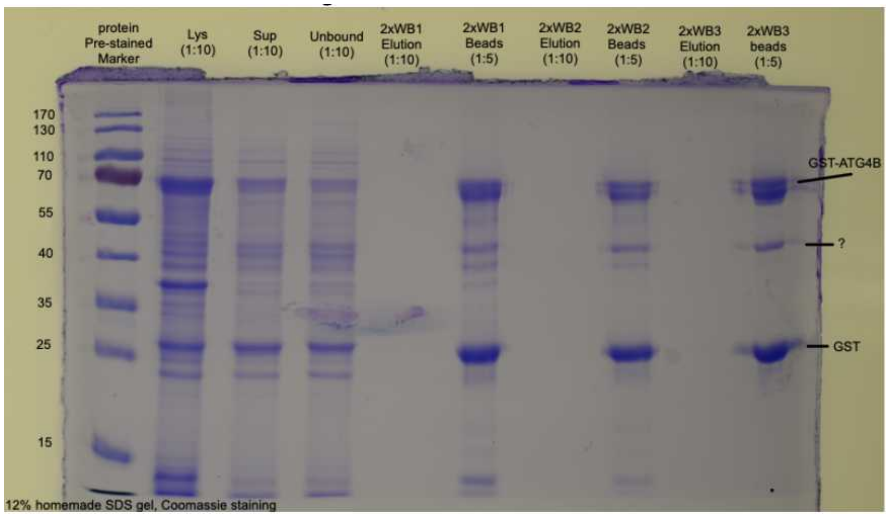

22 Collect sample before cleavage.

23 10h

Incubate beads for **05:00:00** (or less) or **Overnight** rolling at **4 °C** with 1 vial Thrombin (SERVA) to cleave off the GST-tag.

Alternatively, GST tag can be left on and protein is eluted with 50mM reduced L-Glutathione solution in WB3 pH=7.5-8. Elution for GST-tagged proteins is usually performed for 1-2 hours at 4°C, rolling.

24

If cleavage is performed: spin beads down and collect “Elution sample" at **4 °C** (brake=2) in a 5810R centrifuge (Eppendorf).

25 Filter elution through a 0.2µm filter and concentrate down to **2 mL** using a 10kDa cut-off Amicon filter.

26

Keep concentration steps short and pipette regularly to avoid protein precipitation on the filter.

27 In the meanwhile, collect Beads after cleavage sample and check cleavage on a 12% SDS-gel stained with Coomassie Brilliant Blue.

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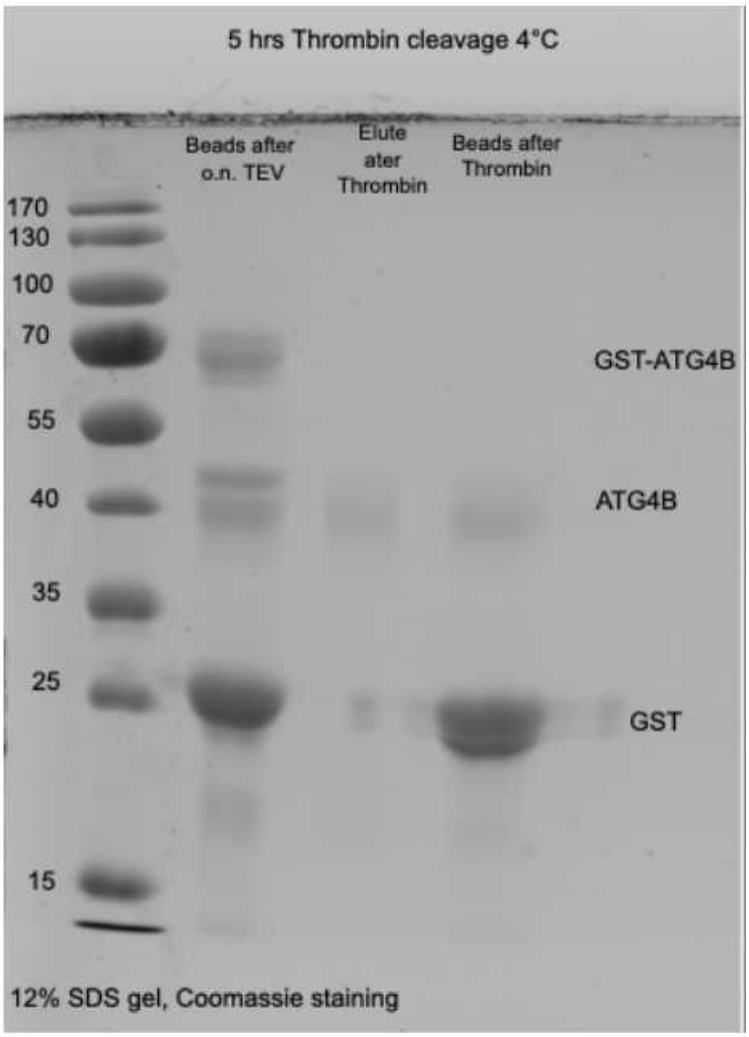

28 Inject **2 mL concentrated protein** onto a S75_16/600 Gel Filtration column pre-equilibrated in Gel Filtration Buffer.

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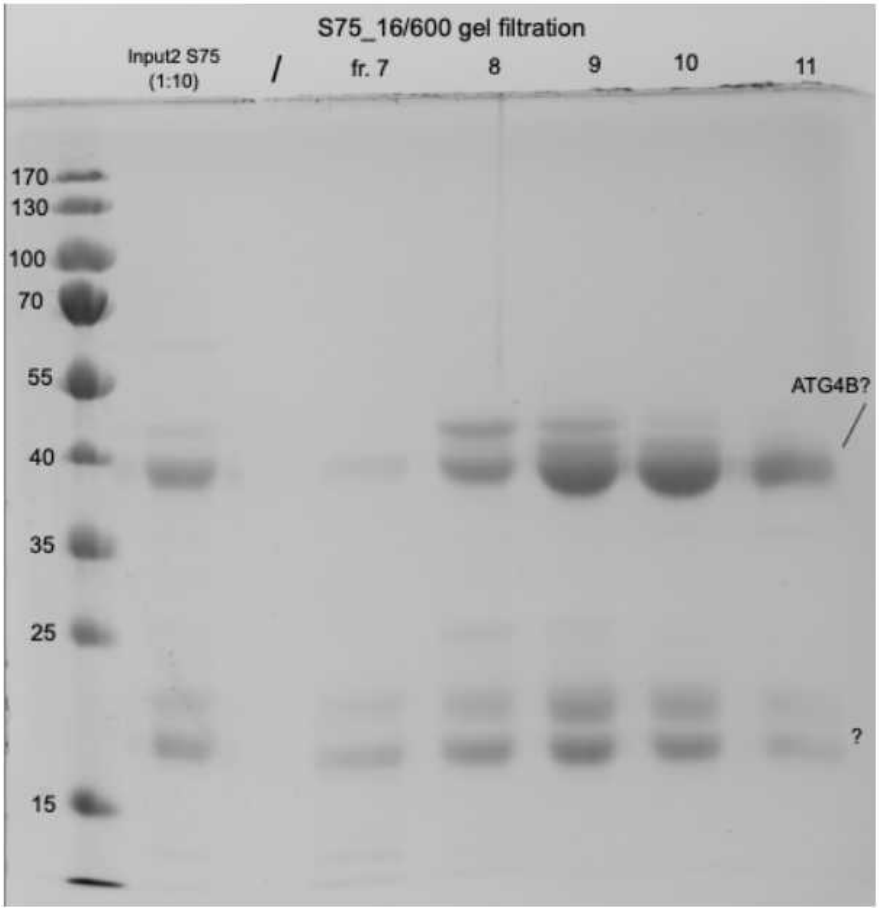
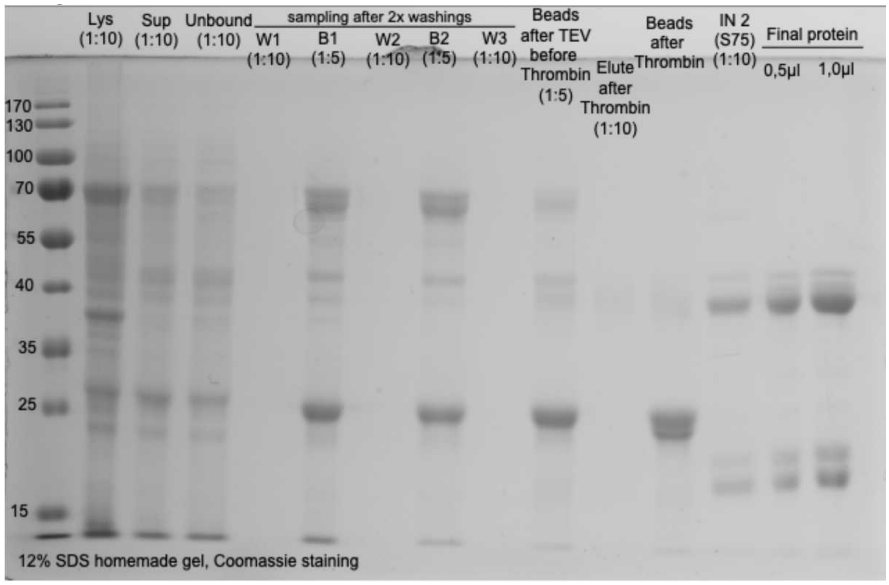


29 Check fractions containing the protein of interest on gel, collect and pool. Concentrate down to reach the desired concentration, flash freeze in liquid nitrogen and store at **-80 °C** until use.

ATG4B, MW=44,3 kDa, Epsilon=65890 M-1 cm-1



Final Gel

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