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Expression and purification GST-tagged ATG13:ATG101 constructs

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DISCLAIMER

ABSTRACT

Expression and purification of GST-tagged ATG13/ATG101 constructs

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Expression 2d 12h 50m

1 Transfect HEK GNTI cells at concentration of 2 × 10^6 cells/ml

2 Dilute PEI with Warm Hybridoma-SFM(1X)

3 In a separate tube, dilute DNA with Hybridoma-SFM(1X)

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4 Add PEI to DNA dilution. Incubate mixture for **00:30:00** at **37 °C** 30m

5 Add mixture to cells. Let cells grow for **48:00:00** 2d

6 Harvest Cells **500 rpm, 4°C, 00:10:00** 10m

7 Wash pellet with cold PBS. Store pellet at -80C until purification or lyse immediately

Purification 2d 12h 50m

8 Resuspended pellet in lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM $MgCl_2$, 1 mM TCEP, 5 mM EDTA, 10% Glycerol) with 1% Triton X-100 and protease inhibitor cocktail (Thermo Scientific, Waltham, MA)

9 Clarify lysate for **17000 rpm, 4°C, 00:35:00** 35m

10 Wash GST Sepharose 4B resin into lysis buffer (without Triton)

11 Load supernatant onto resin using a gravity column setup

12 Rock supernatant with equilibrated resin for **01:00:00** at **4 °C** 1h

13 Wash with 5CV lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM $MgCl_2$, 1 mM TCEP, 5 mM EDTA, 10% Glycerol)

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14 Elute with lysis buffer plus 25 mM glutathione for GST resin

15 Concentrate elution and inject onto pre-equilibrated S200 10/30 column (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM $MgCl_2$, 1 mM TCEP, 5 mM EDTA)

16 Pool peak fractions, concentrate, snap freeze, and store at -80C

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