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Expression and purification Twin-STREP-FLAG tagged ATG13:ATG101 constructs

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DISCLAIMER

ABSTRACT

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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.

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) for 15 min

9 Clarify lysate for **17000 rpm, 4°C, 00:30:00** 30m

10 Wash strep-tactin resin (IBA Lifesciences, Germany) into lysis buffer (without Triton). Load clarified lysate onto resin

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

Use glutathione resin for GST tagged proteins
Use amylose resin for MBP tagged proteins
Use Strep-Tactin Sepharose resin for Strep tagged proteins

12 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)

13 Elute with lysis buffer plus 4 mM desthiobiotin for STREP resin

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14 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)

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

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