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Expression and purification ATG13, ATG101 and FOLDON-ATG9A Proteins from HEK293GnTi Cells

Forked from WIPI2d Expression and purification

Adam Yokom[1]

[1]Team Hurley

1 *Works for me* Share

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Adam Yokom

DISCLAIMER

ABSTRACT

Expression and purification from HEK cells of ATG13, ATG101 and FOLDON-ATG9A proteins

PROTOCOL CITATION

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FORK NOTE

FORK FROM

Forked from WIPI2d Expression and purification, lmstrong

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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)

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)

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

10 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

11 Let supernatant Flow thru gravity column

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 elution additive
- 25 mM glutathione for GST resin
- 40 mM Maltose for amylose resin

4 mM desthiobiotin for STREP resin

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