




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

 Forked from [WIP12d Expression and purification](#)[Adam Yokom](#)<sup>1</sup><sup>1</sup>Team Hurley1 *Works for me* Share

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## 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 [WIP12d Expression and purification](#), [lmstrong](#)

#### KEYWORDS

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Expression

2d 12h 50m

- 1 Transfect HEK GNTI cells at concentration of  $2 \times 10^6$  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<sub>2</sub>, 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<sub>2</sub>, 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<sub>2</sub>, 1 mM TCEP, 5 mM EDTA)
- 15 Pool peak fractions, concentrate, snap freeze, and store at -80C