




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🌐 Expression and purification MBP-ATG9 Constructs

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1 Works for me

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ABSTRACT

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

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KEYWORDS

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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 MgCl₂, 1 mM TCEP, 5 mM EDTA, 10% Glycerol) with 1% Triton X-100 and protease inhibitor cocktail (Thermo Scientific, Waltham, MA)

9 Wash Amylose resin with lysis buffer, ~2mL/1 Liter of cells

10 Clarify lysate by centrifugation 🌀 **17000 rpm, 4°C, 00:30:00** 30m

11 Load supernatant flow thru gravity column onto washed Amylose resin

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

13 Wash with 5 column volumes of lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl₂, 1 mM TCEP, 5 mM EDTA, 10% Glycerol)

14 Elute with lysis buffer plus 40 mM Maltose for amylose resin

- 15 Concentrate elution and inject onto pre-equilibrated S200 10/30 column (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl₂, 1 mM TCEP, 5 mM EDTA)
- 16 Pool peak fractions, concentrate, snap freeze, and store at -80C