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Expression and purification MBP-ATG9 Constructs

Adam Yokom[1]

[1]Team Hurley

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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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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)

30m

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

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 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 $MgCl_2$, 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 $MgCl_2$, 1 mM TCEP, 5 mM EDTA)

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