

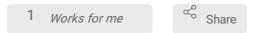


Jul 11, 2022

Expression and purification MBP-ATG9 Constructs

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This protocol is published without a DOI.



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ABSTRACT

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

PROTOCOL CITATION

Adam Yokom 2022. Expression and purification MBP-ATG9 Constructs. **protocols.io**

https://protocols.io/view/expression-and-purification-mbp-atg9-constructs-cc52sy8e

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KEYWORDS

ASAPCRN



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CREATED

Jul 11, 2022

LAST MODIFIED

Jul 11, 2022

PROTOCOL INTEGER ID

66458

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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 8 37 °C

30m

5 Add mixture to cells. Let cells grow for **48:00:00**

2d

6 Harvest Cells **3500 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 **317000 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

- 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