



Jul 20, 2022

# Expression and purification Twin-STREP-FLAG tagged ATG13:ATG101 constructs

Adam Yokom<sup>1</sup>, Xuefeng Ren<sup>1</sup><sup>1</sup>Team Hurley

1 Works for me

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[dx.doi.org/10.17504/protocols.io.yxmvmn4yng3p/v1](https://dx.doi.org/10.17504/protocols.io.yxmvmn4yng3p/v1)

Adam Yokom

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## ABSTRACT

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## DOI

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## PROTOCOL CITATION

Adam Yokom, Xuefeng Ren 2022. Expression and purification Twin-STREP-FLAG tagged ATG13:ATG101 constructs. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.yxmvmn4yng3p/v1>



## KEYWORDS

ASAPCRN

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#### CREATED

Jul 11, 2022

#### LAST MODIFIED

Jul 20, 2022

#### PROTOCOL INTEGER ID

66460

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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) for 15 min
- 9 Clarify lysate for 🌀 **17000 rpm, 4°C, 00:30:00** 30m
- 10 Wash strep-tactin resin (IBA Lifesciences, Germany) into lysis buffer (without Triton). Load clarified lysate onto resin
- 11 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
- 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 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