



Jul 20, 2022

Expression and purification GST-tagged ATG13:ATG101 constructs

Adam Yokom¹, Xuefeng Ren¹¹Team Hurley

1 Works for me

Share

dx.doi.org/10.17504/protocols.io.n92ldzx59v5b/v1

Adam Yokom

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

Expression and purification of GST-tagged ATG13/ATG101 constructs

DOI

dx.doi.org/10.17504/protocols.io.n92ldzx59v5b/v1

PROTOCOL CITATION

Adam Yokom, Xuefeng Ren 2022. Expression and purification GST-tagged ATG13:ATG101 constructs. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.n92ldzx59v5b/v1>



KEYWORDS

ASAPCRN

LICENSE

———— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jul 11, 2022

LAST MODIFIED

Jul 20, 2022

PROTOCOL INTEGER ID

66459

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](#) is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with [protocols.io](#), can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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 or lyse immediately

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 Clarify lysate for 🌀 17000 rpm, 4°C, 00:35:00 35m
- 10 Wash GST Sepharose 4B resin into lysis buffer (without Triton)
- 11 Load supernatant onto resin using a gravity column setup
- 12 Rock supernatant with equilibrated resin for ⌚ 01:00:00 at 🌡 4 °C 1h
- 13 Wash with 5CV 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 25 mM glutathione for GST 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