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Purification and Crystallization of ATG9 HDIR-ATG101:ATG13 complex

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We use this protocol and it's working

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Disclaimer

Abstract

Purification and Crystallization of ATG9 HDIR (828-839) fused ATG101 (1-198):ATG13 (1-197)

Troubleshooting

Expression

1 Transfect HEK GNTI cells at concentration of 2 × 10^6 cells/ml

2 Dilute PEI with Warm Hybridoma-SFM(1X), In a separate tube, dilute DNA with Hybridoma-SFM(1X)

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

4 Add mixture to cells. Let cells grow for 48:00:00 2d

5 Harvest Cells 500 rpm, 4°C, 00:10:00 10m

6 Wash pellet with cold PBS. Store pellet at -80C until purification or lyse immediately

Purification

7 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)

8 Clarify lysate for 17000 rpm, 4°C, 00:35:00 35m

9 Wash GST Sepharose 4B resin into lysis buffer (without Triton)

10 Load supernatant onto GST Sepharose resin using a gravity column setup

11 Rock supernatant with equilibrated resin for 01:00:00 at 4 °C 1h

12 Wash with 5CV lysis buffer (25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM $MgCl_2$, 1 mM TCEP, 5 mM EDTA, 10% Glycerol)

13 Elute with lysis buffer plus 25 mM glutathione for GST resin

14 Add purified His_6-TEV protein to cleave GST tag at 4°C overnight

15 Dilute 5x into SP Buffer buffer A: 30 mM MES pH 6.0, 3 mM beta-mercaptoethanol

16 Load sample onto a HiTrap SP HP 5 ml column (GE healthcare, Piscataway, NJ)

17 Elute from the SP column on a 70 ml linear gradient from 0–500 mM NaCl in SP buffer A. The cleavage sample was eluted at the buffer conductivity of ~ 25 mS/cm.

18 After each fraction was analyzed by SDS gel. Fractions containing ATG9 HDIR-ATG101:ATG13 were pooled concentrated in Amicon Ultra15 concentrator (MilliporeSigma, Burlington, MA) and exchanged into 25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl2, 1mM TCEP for crystallization.

Crystallization

19 ATG9 HDIR-ATG101:ATG13 complex at 6 mg/ml in 25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl2, 1mM TCEP was used as the protein stock

20 Crystallization was carried out by sitting-drop vapor diffusion using an automated liquid-handling system (Mosquito, TTP LabTech, UK) at 288 K in 96-well plates

21 The protein solution was mixed with the reservoir buffer composed of 0.1 M HEPES pH 7.5, 0.2 M NaCl, 12% PEG8000 with a ratio of 1:1

22 Crystal trays were checked daily using a light microscopy for crystal growth

23 Large crystals were obtained in 2–4 days. Crystals were cryo-protectedin 28% glycerol/reservoir buffer and frozen in liquid N_2