



Jul 08, 2022

© Crystallization of ATG9 HDIR-ATG101:ATG13 complex

Adam Yokom¹

¹University of California, Berkeley



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ABSTRACT

Crystallization protocol for the ATG9 HDIR-ATG101:ATG13 complex

PROTOCOL CITATION

Adam Yokom 2022. Crystallization of ATG9 HDIR-ATG101:ATG13 complex. **protocols.io**

https://protocols.io/view/crystallization-of-atg9-hdir-atg101-atg13-complex-ccxmsxk6

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CREATED

Jul 08, 2022



LAST MODIFIED

Jul 08, 2022

PROTOCOL INTEGER ID

66253

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Crystallization

- 1 ATG9 HDIR (828-839) fused ATG101 (1-198):ATG13 (1-197) complex was concentrated to 6 mg/ml in 25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl2, 1mM TCEP
- 2 Crystallization was carried out by sitting-drop vapor diffusion using an automated liquidhandling system (Mosquito, TTP LabTech, UK) at 288 K in 96-well plates
- 3 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
- 4 Crystal trays were checked daily using a light microscopy for crystal growth
- 5 Large crystals were obtained in 2–4 days. Crystals were cryo-protectedin 28% glycerol/reservoir buffer and frozen in liquid N₂