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Protocol status: Working
We use this protocol and it's working

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Sample vitrification and cryo-EM data acquisition

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ABSTRACT

Sample vitrification and cryo-EM data acquisition

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Sample vitrification

1 Glow-discharge the grids at 25 mA for 30 sec with PELCO easiGlow system (Ted Pella)

2 Apply 3 µl of protein solution to the grid:
QUANTIFOIL R1.2/1.3 mesh 300 (Electron Microscopy Sciences), or
QUANTIFOIL R2/1 mesh 300 (Electron Microscopy Sciences)

3 Vitrify the sample with a Vitrobot cryo-plunger (Thermo Fisher Scientific)

4 (Optional) Add 0.05%(w/v) of n-Octyl-Beta-D- Glucopyranoside as a surfactant before vitrification.

cryo-EM data acquisition at 300 kV Titan Krios microscope

5 Collect dataset at a magnification of 81,000x and a corresponding pixel size of 1.05 Å and a defocus range of -0.8 to -2.0 µm. Image stacks contain 50 frames with a total dose of 50 e/Å2.

cryo-EM data acquisition at 200 kV Talos Arctica microscope

6 Collect dataset at a magnification of 36,000x and a corresponding pixel size of 0.5575 Å and a defocus range of -0.8 to -2.0 µm in a super-resolution correlated-double sampling mode. Image stacks contain 50 frames with a total dose of 50 e/Å2.