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Cryo-EM Grid preparation

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details Cryo-EM grid preparation.

Attachments



[852-2198.pdf](#)

36KB

Materials

Materials

- PI3KC3-C1~RAB1A sample with n-Octyl- β -D-Glucopyranoside (OG) at a final concentration of 0.05%
- QUANTIFOIL R 2/1 mesh Cu 300 holey carbon grids
- Vitrobot cryo-plunger (Thermo Fisher Scientific)
- Cryo-EM microscope (300 kV Titan Krios with X-FEG and energy filter)
- K3 Summit direct electron detector (Gatan)
- SerialEM software
- Liquid nitrogen
- Humidity control system



Grid Preparation

1m

- 1 Freshly glow-discharge the QUANTIFOIL R 2/1 mesh Cu 300 holey carbon grids using a glow-discharge system (PELCO easiGlow) at 25 mA current for  00:01:00 .

1m

Sample Application

- 2 Mix the PI3KC3-C1~RAB1A sample with 0.05% n-Octyl- β -D-Glucopyranoside (OG) to achieve the desired final concentration.
- 3 Apply a small volume (typically  3-4 μ L) of the sample onto the glow-discharged side of the grids.

Vitrification

3s

- 4 Load the grids with the sample into the Vitrobot cryo-plunger.
- 5 Set the Vitrobot to the following conditions:
 - 5.1 Maintain 100% humidity inside the chamber.
 - 5.2 Set the temperature to  4 °C .
 - 5.3 Wait for  00:00:03 .
 - 5.4 Blot using a blotting force of -15 (may need calibration depending on your particular instrument) .

3s

Data Acquisition Setup

- 6 Prepare the Cryo-EM microscope (300 kV Titan Krios) for data acquisition.



- 7 Used X-FEG and an energy filter set to 20 eV.

Data Collection Parameters

- 8 Use SerialEM software for automated data collection.
- 9 Set the following parameters for data collection:
 - Magnification: 81,000x.
 - Super-resolution pixel size: 0.525 Å.
 - Defocus range: -0.8 to -2.2 micrometers.
 - Collect 50-frame image stacks.

Data Collection

- 10 Initiate the data collection process using the specified parameters.
- 11 Monitor the data acquisition progress and ensure that the images are being recorded.

Cumulative Dose Control

- 12 Each movie has a cumulative electron dose of 50 e/Å².