ANTCRYO TECHNICAL NOTE · TN-001

Grid Preparation Protocol: Leica EM GP2 Plunge Freezer

Product	ANTcryo amorphous Ni-Ti alloy support films (holey films on standard TEM grids)
Instrument	Leica EM GP2 plunge freezer
Version	1.1
Date	2026-01-01
Status	Released

> This document is part of the ANTcryo technical documentation series. It combines standard cryo-EM grid preparation practice (as documented for Quantifoil holey carbon grids and the Leica EM GP2) with ANTcryo-specific recommendations. Sources are cited in brackets; ANTcryo-specific notes are marked ANTcryo. Parameters marked "starting point" should be optimised for the individual sample.

Revision History

Version	Date	Changes
1.1	2026-01-01	Source verification: removed unverifiable citations, added verified references, corrected sample volume and chamber temperature data
1.0	2025-01-01	Initial release

1. Materials

- ANTcryo grids (film side is the dull, non-reflective side) ANTcryo
- Glow discharge / plasma cleaner (e.g. Gatan Solarus, PELCO easiGlow, Quorum GloQube Plus, Harrick Plasma)
- Leica EM GP2 plunge freezer
- Liquid nitrogen (LN_2); ethane, or a 63/37 ethane/propane mixture
- Blotting paper (pre-punched Whatman No. 1 or 541)
- Leica GP2 tweezers; fine grid-handling tweezers
- Grid storage boxes; cryo gloves and face shield

2. Grid Storage and Handling

- Store grids in the original grid box, **dark, cool, low humidity**; use within 2 years of production to guarantee surface quality. ANTcryo
- In humid conditions, keep grids sealed in a dry container and take them out ~30 min before use to equilibrate to room temperature. ANTcryo
- Inspect grids under a light microscope before use: grids are usable as long as the film is not visibly torn and the hole pattern is intact, even if the grid was briefly blown off by a gas flow. ANTcryo
- Special note: ANTcryo For Cell grids (MS01/MS03) are shipped pre-hydrophilised and can be used directly; after long storage, give them a short plasma treatment first. ANTcryo

3. Glow Discharge (Hydrophilisation)

Glow discharge renders the film surface hydrophilic, which is required for even ice formation and for the sample to stay in the holes. Freshly glow-discharged grids should be used **within 30 minutes** (hydrophilicity decays with time); some protocols recommend within 15 min. [Wang & Zimanyi 2024; Leica App Note 2017]

3.1 Recommended settings

Place grids **film side up** on a glass slide (or grid holder) in the glow discharge chamber.

Instrument	Settings (industry standard)	ANTcryo recommendation
Gatan Solarus	H_2/O_2 mix, ~5-10 W (typical)	**H_2/O_2, 5 W, 30 s** (start) ANTcryo
PELCO easiGlow	20 mA, 30 s per side [STAR Protoc 2020]	**15 mA, 30-60 s** (start at 30 s) ANTcryo
Quorum GloQube Plus	45 s @ 35 mA [Nat Commun 2025]	30-60 s @ 25 mA (start)
Fischione 1070	40 W, 120-180 s, 9:1 Ar:O_2, 21 mTorr [Quantifoil HexAuFoil official]	30-60 s @ 25 mA (start)

3.2 ANTcryo-specific notes

- The amorphous Ni-Ti alloy withstands glow discharge well; **time and intensity can be increased if needed**. As a reference, gold-film grids are commonly treated for ~120 s [Wang & Zimanyi 2024]. ANTcryo
- For a **new batch, first run 2-3 grids** to probe the best settings before committing the sample. ANTcryo
- Common practice for SPA (PELCO easiGlow): air glow discharge at 20-30 mA for 20 s on carbon grids, 120 s on gold grids, and 5 s on ultrathin carbon-coated grids. [Wang & Zimanyi 2024]

4. Leica EM GP2 Setup

Instrument SOP follows the published Leica EM GP2 operating procedure. [Leica GP2 SOP, cryoemcenters.org; Leica EM GP2 Application Note “Plunge Freezing of Microtubules”]

4.1 Environmental chamber

Parameter	Typical SPA setting	Notes
Chamber temperature	**4-10 °C** (SPA typical; up to 25 °C reported)	4 °C for very sensitive samples [Leica App Note 2017; cryoemcenters.org SOP]
Relative humidity	**99 %** (GP2 max)	90-99 %; keep chamber ports closed when not pipetting
Cryogen temperature	~ -183 °C	ethane, or 63/37 ethane/propane (does not freeze over)
GN_2 flow (cryogen Dewar)	100 %	

4.2 Before first use of the day

- Mount fresh blotting paper on the blotting arm; let it equilibrate inside the chamber for **15-20 min** before blotting. Replace blot paper after **every 10 blots or 20 min**, whichever comes first. [Leica GP2 SOP]

- Fill the humidifier with distilled water (~60 ml); fill LN_2 into the freezing chamber; condense ethane into the cryogen cup once the chamber is cold (below about -175 °C). [Leica GP2 SOP]
- Adjust horizontal/vertical blot positions with a blank grid loaded on the tweezers, then run one test blotting cycle.

4.3 Blotting parameters on the GP2 (difference vs Vitrobot)

The GP2 has **no "blot force" dial**; blotting is controlled by **blot time + blot position** (the tweezers/blot arm positions are set once, and the unique infrared blotting sensor adds a fine advance of the paper toward the drop).

- Starting point for ANTcryo grids: **blot time 2.5-4 s**, sensor advance +1.4 mm, blot from the film side. ANTcryo — extrapolated from our Vitrobot settings below; not a separately published GP2 value
- The ANTcryo film tolerates blotting well; in Vitrobot units our standard is blot time 3 s, force 2, one blot, which routinely gives thin, even ice (see Section 6). On the GP2, start at 2.5-4 s and screen a couple of grids to tune ice thickness. ANTcryo

5. Sample Application

- Use **3-4 µL** of sample per grid (typical). The GP2 blotting sensor is designed for ~4 µL; 3 µL works reliably. [VIB BECM GP2 SOP; Nat Commun 2025]
- Apply the drop onto the film side; do not touch the film with the pipette tip.
- Concentration starting points ANTcryo:
  - **Apoferritin: 1-1.5 mg/ml** as a standard reference (screen 2-3 concentrations if needed)
  - For scarce/challenging samples (e.g. membrane proteins): the low-adsorption Ni-Ti surface keeps more of the precious sample in the holes; test a small grid set before scaling up. ANTcryo
- Optional: incubate the drop on the grid for 20-30 s in the humidified chamber before blotting (adsorption/incubation improves particle density for some samples). [Nat Commun 2025; Leica App Note]

6. Expected Ice Behaviour (ANTcryo)

- The smooth, uniformly hydrophilic Ni-Ti film favours **even ice of 20-80 nm**, reducing thick/thin ice gradients. ANTcryo
- Under our standard Vitrobot settings (blot 3 s / force 2 / 1 blot), the success rate for ~20 nm ice is notably higher than on conventional carbon films. On the GP2, expect a similar window; verify with your first grids. ANTcryo

7. Plunge Freezing and Transfer

1. Load the glow-discharged grid on the Leica GP2 tweezers (film side facing the side you will pipette onto), click the tweezers into the plunge rod.
2. Press "LOAD SPECIMEN"; apply the sample through the side port.
3. Press "BLOT" (auto-plunge after blotting if enabled); the grid plunges into liquid ethane.
4. After plunging, immediately transfer the grid, while submerged in LN_2, from the ethane cup into an open slot of the grid box in the transfer container.
5. Store grid boxes in LN_2 until screening/data collection.

8. Troubleshooting

Symptom	Likely cause	Remedy
Wavy/corrugated square edges	Uneven glow discharge; excessive blot force or blot time	Retune glow discharge; reduce blot time / relax blot position ANTcryo
Droplets / thick ice	Humidity too low, or blotting too weak	Check chamber humidity (99 %); increase blot time ANTcryo
Particles stuck to film, few in holes	Film surface not hydrophilic enough	Re-glow discharge (longer time); check the 30-min window ANTcryo
Grid damage after blotting	Blot pressure too high; bent grids	Some edge damage is normal and does not affect data (1-2 central squares suffice); discard bent grids ANTcryo
Poor ice reproducibility between sessions	Humidity/water contamination	Replace blot paper; check humidifier fill; keep ports closed

9. Sources

- Leica Microsystems, “Plunge Freezing of Microtubules” EM GP2 Application Note (2017): Quantifoil R2/2 on 400-mesh Cu, glow discharge 45 s, 25 °C / 95 % RH, blot 1.3 s +1.75 mm.
- Leica EM GP2 Standard Operating Procedure, cryoemcenters.org: blot paper equilibration 15-20 min, humidifier ~60 mL, ethane condensation below -175 °C, blot paper replaced every 10 blots / 20 min, chamber 4-25 °C / 0-99 % RH, GN_2 flow 100 %.
- VIB BECM (Brussels) Leica GP2 SOP, becm.sites.vib.be: GP2 blotting sensor designed for ~4 µL sample; 3 µL works reliably; Whatman 1 paper.
- STAR Protocols 1, 100150 (2020), human 26S proteasome: easiGlow 20 mA 30 s per side; Quantifoil R1.2/1.3 200-mesh Cu.
- Kampjut, Steiner & Sazanov, iScience 24:102139 (2021): Quantifoil carbon 0.6/1 or 1.2/1.3 grids (or UltrAuFoil gold 0.6/1), glow discharge in air 2 min @ 7×10^{-1} mbar, ~25 mA; Vitrobot blot force 25, blot 4-10 s.
- Wang & Zimanyi, Acta Cryst F 80:74-80 (2024): easiGlow 20-30 mA 20 s (carbon), 20-30 mA 120 s (gold), 10 mA 5 s (ultrathin carbon); use grids within 30 min of glow discharge.
- Nat Commun (2025), Chromera velia PSI structure (10.1038/s41467-025-67637-0): GloQube Plus 45 s @ 35 mA, Quantifoil R1.2/1.3 Cu 300, 3 µL sample, 30 s incubation, 4 °C / 100 % RH.
- ANTcryo support FAQ (nanodim-world.com/support, “Sample Preparation & Handling”); ANTcryo Product Data.

This technical note is provided as technical support. Parameters are starting points; final settings depend on the individual sample and instrument. For batch-specific questions, contact service@nanodim-world.com.