

Best practices for preparing grids using VitroEase Apoferritin Protein Standard

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Preparing good cryo-electron microscopy (cryo-EM) grids starts with a stable protein sample. Thermo Scientific[™] VitroEase[™] Apoferritin Standard is specifically designed for use in cryo-EM. In this application note, we share the recommended settings for preparing Apoferritin cryo-EM grids using the Thermo Scientific[™] Vitrobot[™] Mark IV System and make suggestions to prevent common pitfalls.

Preparing Apoferritin grids with the Vitrobot Mark IV System

Workflow		
	Connect and fill the Vitrobot System's humidifier	
\checkmark	Place the filter papers on the blot pads	
\checkmark	Adjust vitrification settings using the recommended settings in Table 1	
\checkmark	Treat the EM grids with glow discharge or plasma	
	Thaw one Apoferritin aliquot by hand, keep on ice, and add DTT to a final concentration of 1 mM	
	Prepare liquid ethane according to the Vitrobot System's user manual	
\sim	Plunge freeze the grid with Apoferritin	
	Transfer the grid to the grid box	
	NOTE: Blot time may need to be further optimized.	

Resources for preparing cryo-EM grids

- The <u>Thermo Scientific[™] VitroEase[™] Cryo-EM Training Kit</u> includes everything you need to prepare your first Apoferritin grids
- **EM-University** features educational videos on cryo-EM theory and applications

Recommended vitrification settings

Parameter	Value
Grid type	QUANTIFOIL R 1.2/1.3, Cu 200 or 300 mesh
Applied sample volume	3 µL
Blot time	4 s
Relative humidity	100%
Temperature	4°C
Blot force	0
Wait time	0 s
Drain time	0 s

Table 1. Recommended vitrification settings for Apoferritin using the Vitrobot Mark IV System

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Precautions

Keep the following points in mind when making Apoferritin grids:

- Thawed Apoferritin aliquots should be kept on ice or at 4°C while setting up and preparing grids with the Vitrobot Mark IV System
- Do not re-freeze Apoferritin aliquots once thawed; use the full aliquot to prepare additional grids and store these for future use
- Add 1mM DTT to the aliquot prior to use (eg. 1 µl of 20mM DTT stock solution and mix by pipetting to one of the ApoF aliquots)
- If aggregation is observed in the tube, centrifuge the sample at 14,000 x g for 10 minutes at 4°C and use only the top layer of the liquid for vitrification
- The VitroEase Apoferritin Standard comes in optimal concentration ranges with ready-to-use aliquots; sample dilution is not recommended



Figure 1. Representative images of grid squares (A, C) and corresponding high-magnification images (B, D) of two different VitroEase Apoferritin Standard batches prepared with the recommended vitrification settings.

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