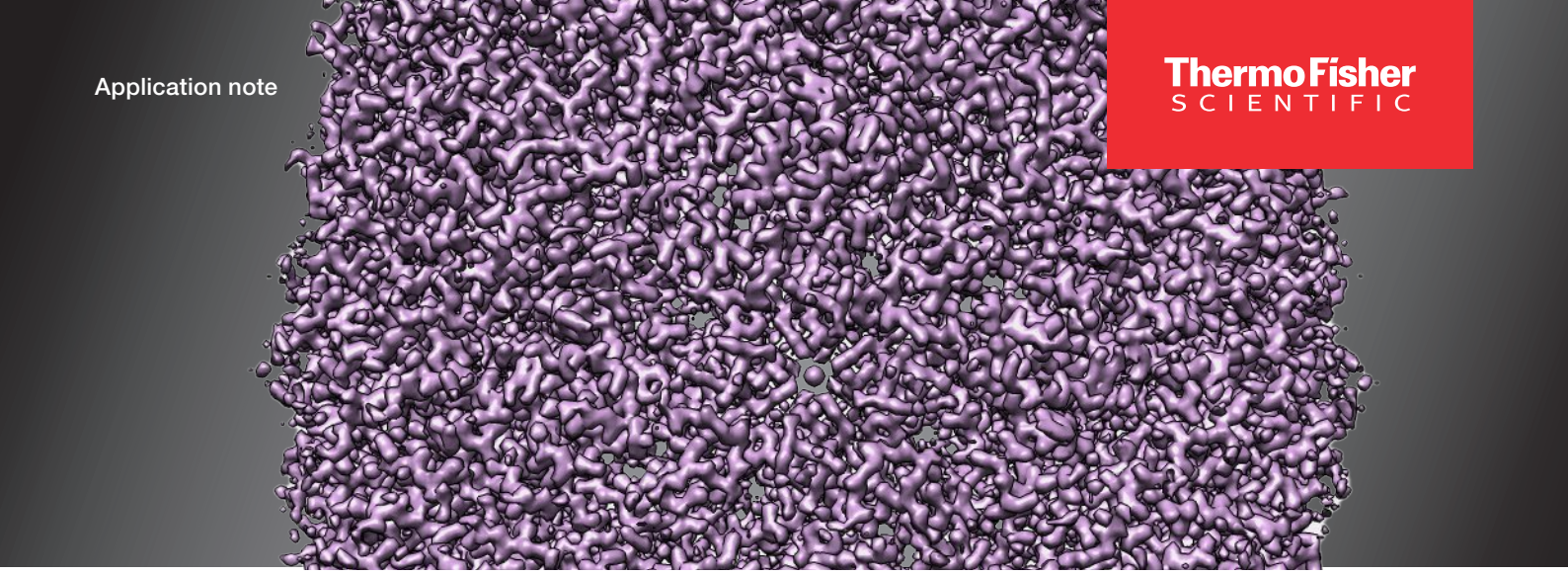
Application note



Best practices for preparing grids using VitroEase Apoferritin Protein Standard

by Dimple Karia, Natalya Dudkina, and Albert Konijnenberg

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
- Place the filter papers on the blot pads
- Adjust vitrification settings using the recommended settings in Table 1
- 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
- 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 Thermo Scientific™ VitroEase™ Cryo-EM Training Kit 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

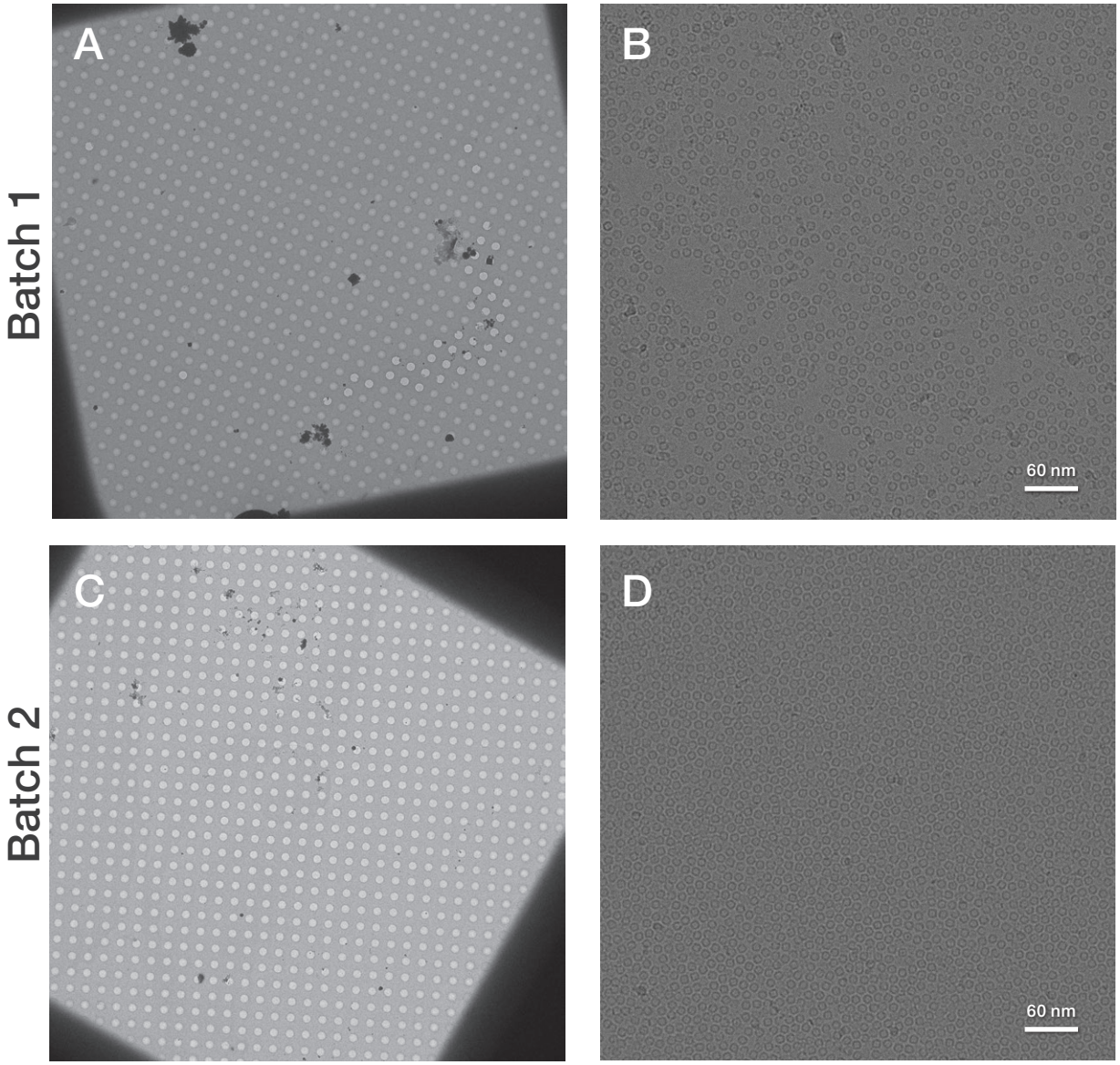

Figure 1. Representative images of grid squares (A, C) and corresponding high-magnification images (B, D) of two different VitroEase Apoferritin Standard batches prepared with the recommended vitrification settings.

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