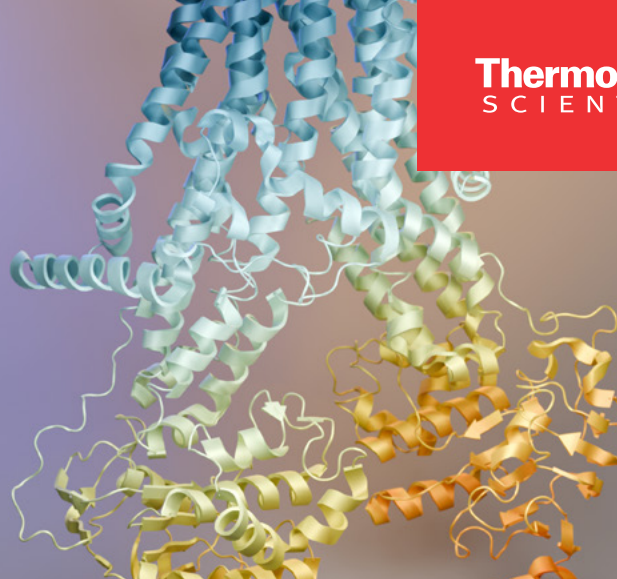




Grid-preparation best practices using the VitroEase ABCC4 Membrane Protein Standard

Cryo-electron microscopy (cryo-EM) has had a significant impact on the structural elucidation of membrane proteins, yielding high-resolution 3D structures of previously unobtainable targets for drug discovery.

The Thermo Scientific™ VitroEase™ ABCC4 Membrane Protein Standard is specifically designed and tested for use in cryo-EM. In this application note, we share the recommended settings for the preparation of ABCC4 cryo-EM grids using the Thermo Scientific™ Vitrobot™ Mark IV System, along with some general guidelines for working with membrane protein samples.

Preparing ABCC4 grids with the Vitrobot Mark IV System

1. Connect and fill the Vitrobot System humidifier
2. Place the filter papers on the blot pads
3. Adjust vitrification parameters using the recommended settings in Table 1
4. Treat the EM grids with glow discharge or plasma
5. Thaw one aliquot of the VitroEase ABCC4 Standard by hand and keep the sample on ice
6. Prepare liquid ethane according to the Vitrobot System user manual
7. Plunge freeze the grid with the VitroEase ABCC4 Standard
8. Transfer the grid to a grid box

Resources for preparing cryo-EM grids

- The [VitroEase Cryo-EM Training Kit](#) includes everything you need to start preparing your first cryo-EM grids
- The Cryo-EM University (thermofisher.com/em-university) features educational videos on cryo-EM theory and applications
- The sample preparation webpage (thermofisher.com/emsampleprep) offers instructional videos on how to prepare and operate the Vitrobot Mark IV System

Table 1. Recommended vitrification settings

Parameter	Value
Grid type	QUANTIFOIL R1.2/1.3, Cu 200 or 300 mesh
Applied sample volume	3 µL
Blot time	6 seconds
Relative humidity	100%
Temperature	4°C
Blot force	0
Wait time	0 seconds
Drain time	0 seconds

Note: Blot times may need to be further optimized.

General considerations

When working with the VitroEase ABCC4 Membrane Protein Standard, please consider the following:

- The standard is provided in a 50 mM Tris-HCl buffer at pH 8.5, supplemented with 150 mM KCl, 2 mM DTT, and 2 mM MgCl₂, containing 0.005% (w/v) GDN. When diluting the sample, make sure that the detergent concentration remains the same to prevent the protein from crashing out of solution.
- The standard comes in 15 µL aliquots, enough for the preparation of 5 grids per aliquot. As with any membrane protein, do not re-freeze unused sample from an aliquot, as the protein will become unstable.
- The ABCC4 membrane protein comes in ready-to-use aliquots with optimized concentration ranges — sample dilution is not recommended.

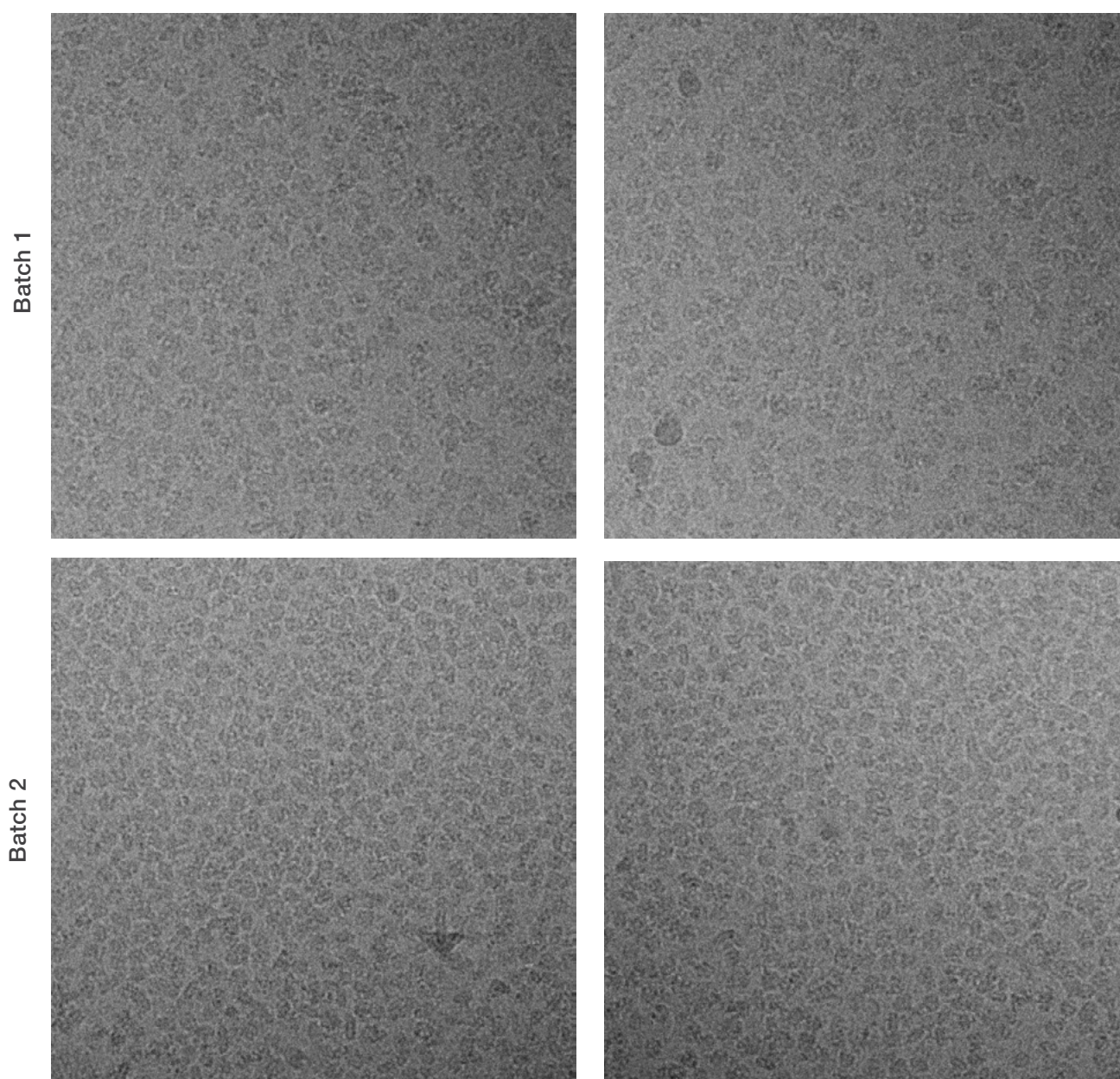


Figure 1. Representative images of two different VitroEase ABCC4 Membrane Protein Standard batches prepared with the recommended vitrification settings listed in Table 1.

Learn more at thermofisher.com/emsampleprep

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