DATASHEET

Requirements for Preparation of Cryo-EM Samples

A vital step in the cryo-EM workflow

The cryo-EM sample is typically a vitrified suspension of biological material consisting of proteins, protein complexes, viruses or other macromolecules.

As sample preparation is a vital step in the cryo-EM workflow, it is important to have a good understanding of the sample preparation steps and to possess the right set of instruments.

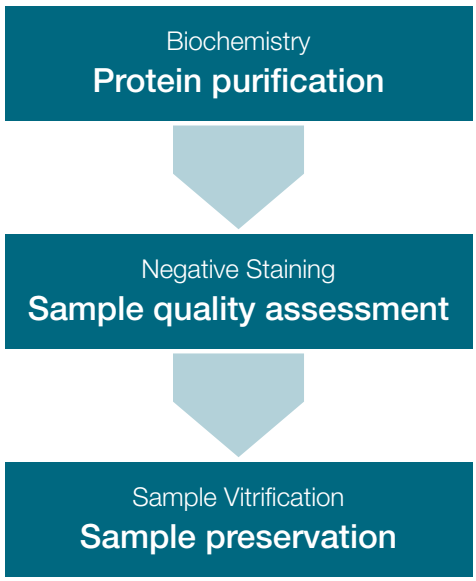
- Thermo Fisher Scientific provides a workflow assistance app (iPad) to guide users through the main steps of cryo-EM sample preparation.
- Many different protocols for cryo-EM sample preparation have been developed and published (reference list is provided below). Choosing the best protocol depends primarily on the type of sample material.
- Our customer success manager provides some practical recommendations for sample preparation laboratories.
- The set of instruments required to prepare the cryo-EM sample is provided below.

The studied sample must fulfill the following requirements:

- Very high (>99%) sample purity (a single band in SDS-PAGE gel)
- Minimal compositional heterogeneity (a single peak in SEC chromatogram)
- Minimal conformational heterogeneity (i.e. lock in one or only a few different states)

Sample preparation steps

To prepare a cryo-EM sample suitable for high-resolution data collection, the following steps are typically followed:



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Protein purification

Although the single particle analysis workflow can alleviate partial heterogeneity in the sample via 3D classification procedures, biochemical purification of the sample material to obtain a solution of isolated target proteins is required.

Cryo-EM samples are typically prepared using several microliters of protein solution at a concentration ranging between 50 nM and 5 µM, depending on the sample, EM grids and conditions used.

The studied biological specimen should remain active in the *in vivo* optimized conditions (buffer composition etc.) for structural studies. A suitable biochemical or other functional assay might also be exploited to test the activity of the protein.

Typical biochemical and biophysical methods used for assessment of protein sample composition and homogeneity are:

- Polyacrylamide gel electrophoresis (SDS-PAGE)
- Size exclusion chromatography (SEC)
- Dynamic light scattering (DLS)

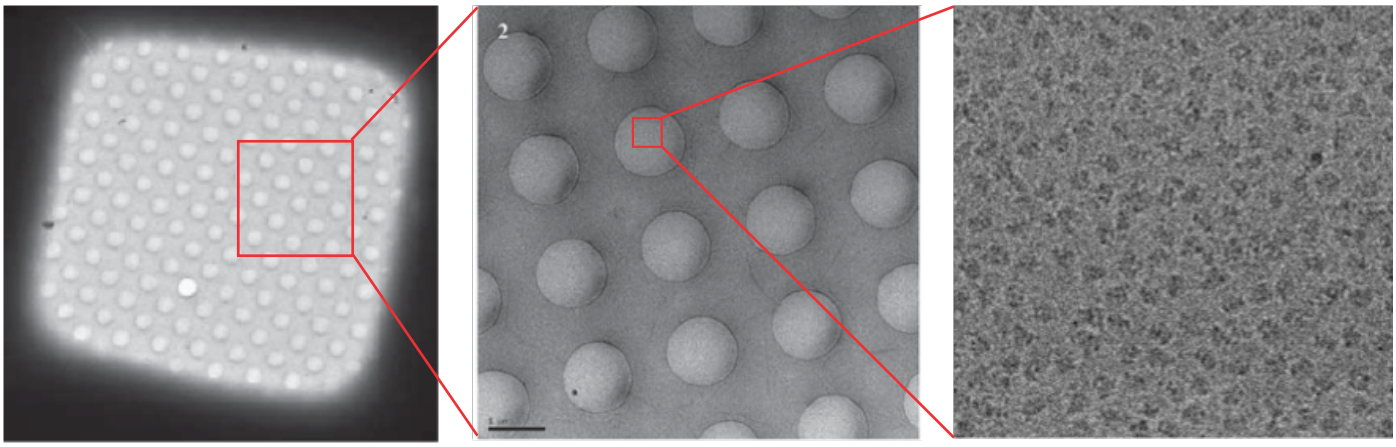
Typically, most of these techniques would be available in regular biochemical labs. Thus, little or no additional investment would be required for the biochemical part of cryo-EM sample preparation.

Sample quality assessment

An easy and straightforward method to assess the quality of purified biological samples at the microscopic scale is negative-stain electron microscopy. The objective of this screening is to qualitatively assess particles' compositional and conformational homogeneity. This assessment can be done only at the microscopic scale.

Often, this assessment of biochemical quality is done on a simple side-entry microscope (for example: Thermo Scientific™ Talos™ L120C TEM or Thermo Scientific™ Talos™ F200C TEM), since screening is usually done one grid at a time, and the actual time spent on the microscope is short.

Alternatively, the screening for biochemical quality can be done at cryogenic conditions, which effectively means it will be combined with the screening for frozen hydrated samples, described in the next section.



Perfect grid square: homogeneous, clean, thin vitreous ice.

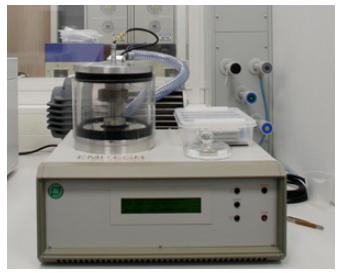
Sample preservation

For compatibility with the electron microscope vacuum, and to lock the individual particles in place, the solution containing the sample material must be frozen. In order to preserve the macromolecular structures undamaged, freezing must happen quickly enough to avoid crystalline ice to occur. This is accomplished by rapid plunging. Then the sample has to be kept at liquid nitrogen temperatures at all times to preserve the amorphous nature of the embedding ice layer and to avoid damage to the biological particles.

This operation produces a frozen hydrated sample, where the individual molecules of the sample are embedded in a very thin layer of amorphous (vitreous) ice and are well distributed within the ice.

The entire procedure can be simplified using semi-automated plungers such as the Thermo Scientific™ Vitrobot™ System. Based on a set of key parameters, such as sample blotting time, blotting force, relative humidity and temperature, this allows for reproducible preparation of vitrified samples at high quality.

Thermo Scientific Vitrobot Mark IV System: state-of-the-art sample preparation unit for cryo-EM



Glow discharge

Thermo Scientific cryo-electron microscopes use patented AutoGrid sample carriers. These AutoGrids are the industry standard for robust and reliable loading and unloading of samples using a robotic sample loader at cryogenic temperatures. AutoGrids also allow flawless interchange of samples between different microscopes in the workflow using designed-in connectivity.

Recommended equipment for cryo-EM sample preparation

Vitrobot Mark IV System	Plunge freezing of grids
Glow discharge unit / plasma cleaner Carbon evaporator	Preparation of grids • Hydrophilicity of the EM grid support film is achieved and controlled by glow discharge or plasma treatment, to optimize the distribution of particles in ice • Optionally provides an additional carbon layer
Polarization optical microscope	AutoGrids preparation • For ensuring that AutoGrids are properly clipped into the autoloader cartridge
Precision tweezers	General manipulation of grids prior to freezing
A small 37°C oven	Accelerated and thorough drying of small tooling
Accessories provided by Thermo Fisher Scientific, as part of the cryo-electron microscope delivery • C-clip insertion tool (4x) • AutoGrid alignment tool • AutoGrid containers • Grid container box • Auto grid assembly workstation	Small tools for assembling and storing AutoGrids
Consumables – a starter set will be provided by Thermo Fisher Scientific as part of the cryo-electron microscope delivery • C-clip rings (AutoGrids) • C-clips • EM grids	Consumables for AutoGrids – this is the industry-standard sample carrier for cryo-EM applications

All equipment, accessories and consumables are commercially available.

Cryo-EM sample preparation can be performed in a standard biochemistry laboratory. The above equipment is recommended for a cryo-EM sample preparation setup.

Reference list:

1. L.A. Passmore, C.J. Russo, , Specimen preparation for high-resolution cryo-EM. Methods in Enzymology (2016), 579: 51-86. doi: 10.1016/bs.mie.2016.04.011

2. Y. Cheng, N. Grigorieff, P. A. Penczek, and T.s Walz, A Primer to Single-Particle Cryo-Electron Microscopy. Cell (2015), 161(3):438-449. doi:10.1016/j.cell.2015.03.050.

3. C.J. Russo, L.A. Passmore. Ultrastable gold substrates for electron cryomicroscopy. Science (2014), 46(6215):1377–1380. doi: 10.1126/science.1259530

4. R. F. Thompson, M. Walker, C. A. Siebert, S. P. Muench, N. A. Ranson. An introduction to sample preparation and imaging by cryo-electron microscopy for structural biology. Methods (2016), 100: 3-15. doi: 10.1016/j.ymeth.2016.02.017

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Notes

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