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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.

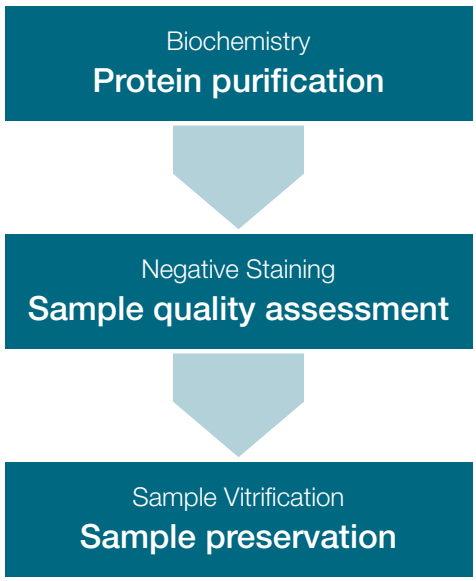
- Thermo Fisher Scientific provides a workflow assistance app (iPad) to guide users through the main steps of cryo-EM sample preparation.
- Many different protocols for cryo-EM sample preparation have been developed and published (reference list is provided below). Choosing the best protocol depends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

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 for vitrification.

The studied biological specimen should remain active and stable* in the *in vitro* optimized conditions (buffer composition, etc.) for structural studies. A suitable biochemical or other functional assay might also be exploited to test the stability and 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)

** Activity is applicable only for samples such as enzymes. For samples such as viruses stability is applicable.*

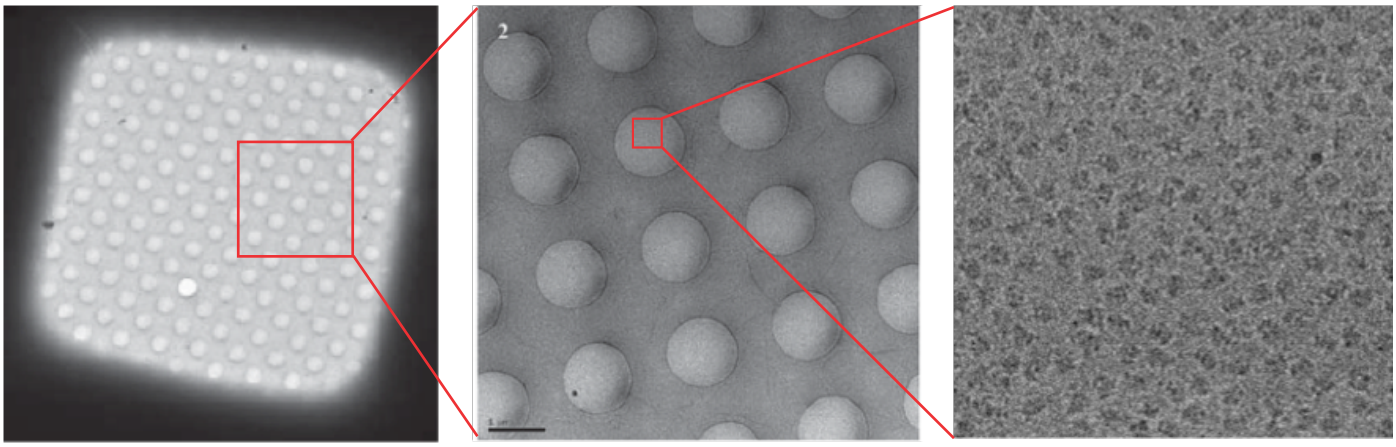
Typically, most of these techniques would be available in regular biochemical labs. Thus, little or no additional investment would be required for the biochemical part of cryo-EM sample preparation.

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 a specimen's 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

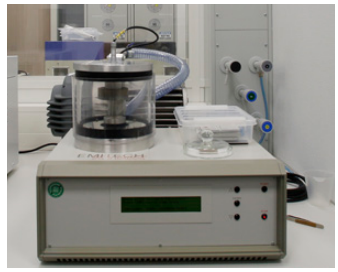
For compatibility with the electron microscope vacuum, and to lock the individual particles in place, as well as reduce radiation damage and increase preservation in a water-like environment, the solution containing the sample material must be frozen. In order to preserve the macromolecular structures undamaged, freezing must happen quickly enough to avoid crystalline ice formation. 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 randomly 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 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.

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



Glow discharge

Cryo-EM sample preparation can be performed in a standard biochemistry laboratory. The equipment listed in the table on the following page is recommended for a cryo-EM sample preparation setup.

Reference list:

1. K. Sader, R. Matadeen, P. Castro Hartmann, T. Halsana and C. Schlichten, **Industrial cryo-EM facility setup and management**. *Acta Crystallographica Section D* (2020) D76, 313–325. doi: org/10.1107/S2059798320002223
2. 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
3. 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.
4. C.J. Russo, L.A. Passmore. **Ultrastable gold substrates for electron cryomicroscopy**. *Science* (2014), 46(6215):1377–1380. doi: 10.1126/science.1259530
5. 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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Recommended equipment for cryo-EM sample preparation		
Device	**Purpose**	**Suggested supplier**
Vitrobot Mark IV System	Plunge freezing of grids	Thermo Fisher Scientific
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	Quorum
Polarization optical microscope	AutoGrid preparation • For ensuring that AutoGrids are properly clipped into the autoloader cartridge	
ESD soft grip tweezers (electrostatic discharge) 15mm, Extra Fine Tips (or equivalent)	General manipulation of grids prior to freezing	Agarscientific
Taylor Wharton LN2 4l dewars	Easy nitrogen pouring	Linde Group or Fisher Scientific
Taylor Wharton LN2 120l tank with connector and cryohose	Liquid nitrogen storage	Linde Group
Explosive cabinet for ethane, including installation and connection to exhaust	Safety cabinet for ethane cylinder	Asecos via Fisher Scientific
Ethane gas cylinder 20l	Source of ethane for grid plunging	Airliquide
Storage dewar Taylor Wharton HC35 with roller base	Long-tern storage for vitrified grids	Linde Group
Grid storage system	Organized storage of vitrified grids; fits into HC35	Subangstrom
Grid storage accessory puck	Organized storage of vitrified grids; fits into Grid Storage Sys.	Subangstrom
Foam dewar	Transfer vitrified grids into pucks	Subangstrom
Taylor Wharton dry shipper CX100 w/ case	Ship vitrified grids	Linde Group
Digital dry bath with block heaters	Dry cryo-tools during grid clipping and loading	Thermo Fisher Scientific
Pipettes 0.5-10ul Eppendorf	Apply sample on a grid	Thermo Fisher Scientific
Drying cabinet	Accelerated and thorough drying of small tooling	Sigmaaldrich
Processing PC with 4GPUs	Calculate reconstructions	Aragorn
Liquid nitrogen tipper	Easy nitrogen pouring into dewars	Boconline
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	Thermo Fisher Scientific
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	Thermo Fisher Scientific

All equipment, accessories and consumables are commercially available.

Find out more at **thermofisher.com/EM-Sales**

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