

**Q1. Do Users need to prepare consumables or standards?**

A1. The facility provides relevant consumables for experiments, users only need to prepare samples, yet can also bring their own copper grids.

**Q2. What type of grids should be used for Cryo-EM? Does ASCEM provide the grids?**

A2. CryoEM only use Quantifoil or holey carbon grids and ASCEM provides both grids, but users need to pay for them.

**Q3. Does ASCEM provide original Vitrobot filter papers?**

A3. Both handmade and factory specifications are provided on site for user to choose freely. The two filter papers had been tested in CryoEM experiments and functioned the same.

**Q4. What solvent should be used for sample preparation?**

A4. Pure water is the best solvent for CryoEM experiments.

- It is recommended to use hydrophilic buffer that most suitable for sample, low-salt concentration, better not to add surfactant, and better no sucrose or glycerol.
- The buffer viscosity will affect the thickness of the ice layer, the more viscous the buffer when making the cryo-grid, the ice layer will be too thick or be empty.

**Q5. Taking liposomes as the example, what should be processed or paid attention to before delivering samples to ASCEM?**

A5. Upon the facility's experience of liposome cases, the sample concentration is most important. The lipid concentration between 1 mg/ml and 10 mg/ml is usually recommended. If the sample concentration is too dilute, it will take a lot of time to search for sample after loading onto cryo-EM. Each sample requires 20 ul in amount.

**Q6. What is the appropriate sample concentration for cryo-EM experiment? Is there any restriction for the sample storage buffer?**

A6. The concentration is mildly related to the sample size in cryo-EM. If the sample size is about 200kDa, then a concentration of 0.5mg/ml could be a start. Viscous buffer will affect more in cryo-grid preparation.

**Q7. How many hours that need to be booked for conducting an experiment on CEM1?**

A7. For a negative staining experiment, up to 2 grids can be observed in an hour. The total hours to be booked depend on user's proficiency in the sample purification. With more confidence, relatively the less samples that need to be prepared and so the shorter the observation time will be.

**Q8. What are the pre-treatments or buffer restrictions for samples doing the negative staining?**

A8. Samples to be negatively stained basically don't require the pre-treatment, but please note the buffer should not be too viscous to avoid affecting the negative staining effect. Users only need to deliver the sample solution one day prior to the booked CEM1 date.

**Q9. Can multiple people be on site together when using instrument?**

A9. The lab needs to maintain the environment with low humidity and constant temperature, the case applicant is expected to be on site for each application case. If necessary, only one more accompany can participate in the experiment, but please inform the manager or staff in advance.

**Q10. Is Wifi available in the lab?**

A10. In order to optimize the low electromagnetic wave experimental environment and control information security, the facility does not provide Wifi to users, but campus Wifi is available in the Control Room. If it is necessary to contact any outside person during the experiment time, please seek assistance from manager or staff to use the online communication software via the support PC in the lab.