

# C-flat™

## Advanced Holey Carbon Film for High-Resolution TEM

GroEL, 7A resolution

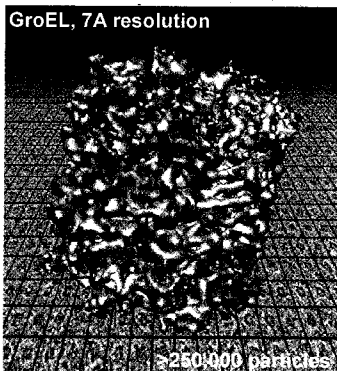


Image courtesy of Scott Stagg and Mike Pique,  
NRAMM, The Scripps Research Institute

Protochips, Inc.

### Cryo Preparation using C-flat™

C-flat™ is a holey carbon support film, manufactured using a patent pending semiconductor-based technology without plastics, resists or other soft materials. As a result, the carbon films are flat, uniform and free of residues or plastics. C-flat™ is designed to be an "out of the box" solution, and should require minimal sample preparation. **Extensive plasma cleaning is not needed, and could potentially thin the carbon, making it too fragile for blotting or freezing.**

**If you are using C-flat™ for the first time, it is recommended that no plasma preparation be used initially.** As with any carbon film, plasma preparation is sometimes necessary to make the surface more hydrophilic. If your initial results dictate making the films more hydrophilic, below are some guidelines for preparation using several common systems.

#### Fischione Model 1020

25% Oxygen / 75% Argon

Use 5 grid holder and dampening shield  
Plasma clean grids for 10-30 seconds

### C-flat™ Holey Carbon Films

#### Product Description

C-flat™ is an ultra-flat, carbon support film with a regular array of  $\mu\text{m}$ -sized holes and is perfect for many applications in high-resolution cryo electron microscopy (EM). C-flat™ is shipped with the **carbon side facing the tab on the grid box.**

#### Product Information

C-flat™ is manufactured with a patent pending process that does not use plastic films or resins. As a result **C-flat™ does NOT contain any plastic residues** that would require excessive cleaning in plasma, solvents, or acids prior to use. See below for additional sample preparation information.

#### Single Particle Analysis & Electron Tomography

Numerous researchers have reported that the ultra-flat surface of C-flat™ leads to uniform particle distribution within the hole area and this optimal particle distribution results in superior data being collected.

*It is recommended that the dampening shield be used when cleaning C-flat™ using the Fischione Model 1020 plasma cleaner. The shield will dampen the effect of the plasma, reducing the erosion rate of the carbon while allowing the film to become more hydrophilic.*

#### Gatan Solarus™

25% Oxygen / 75% Argon

Place grids on a support (e.g. glass slide)

Set slide in the bottom of the chamber

Set RF power to 25 watts

Plasma clean grids for 10-20 seconds

#### Glow discharge

These systems vary widely depending on the manufacturer. Typically, keep the glow from the plasma dim and the clean time approximately 10-30 seconds.

Recommended settings for plunge freezing:

#### Vitrobot™

Temperature: 4°C

Humidity: 100% (can vary between 90-100%)

Blot time: 3-5 seconds

Volume on Grid: 3 $\mu\text{L}$  (can vary)

Drain time: 0 seconds

Offset: 0 for regular samples, -1 for viscous

#### Pricing & Availability

C-flat™ is sold in quantities of 25, 50 and 100 grids and is available in a wide variety of patterns on 200 and 400 mesh Cu TEM grids:

1.0 $\mu\text{m}$  hole / 1.0 $\mu\text{m}$  space

1.2 $\mu\text{m}$  hole / 1.3 $\mu\text{m}$  space

2.0 $\mu\text{m}$  hole / 0.5 $\mu\text{m}$  space

2.0 $\mu\text{m}$  hole / 1.0 $\mu\text{m}$  space

2.0 $\mu\text{m}$  hole / 2.0 $\mu\text{m}$  space

2.0 $\mu\text{m}$  hole / 4.0 $\mu\text{m}$  space

4.0 $\mu\text{m}$  hole / 1.0 $\mu\text{m}$  space

4.0 $\mu\text{m}$  hole / 2.0 $\mu\text{m}$  space

multi-hole and space

#### Custom Solutions

The 2/1, 2/2, 4/2 and multi-hole patterns are available in both our standard and double thickness carbon films. C-flat™ is a fully customizable product, including grid metal and mesh size. Please contact us at [cflat@protochips.com](mailto:cflat@protochips.com) or [sgkck@aol.com](mailto:sgkck@aol.com) to discuss your custom C-flat™ needs.

*When using the Vitrobot™, it is recommended that the filter paper be changed regularly (generally after freezing 4-5 grids or 10 minutes, whichever comes first.) The filter paper can become saturated in the high humidity environment of the chamber.*

### Sample Preparation Solutions

#### Working with Viscous Samples

Generally, lowering the volume of solution on the grid can help to eliminate the need for multiple blots, which can damage the carbon film. As little as 1 $\mu\text{L}$  of solution can cover a 3mm grid area if the pipette tip is used to spread the drop, but reducing the volume to 1.5 or 2.0 $\mu\text{L}$  will help as well. Once the sample is on the grid, it should be blotted within a few minutes before further evaporation occurs. If a Vitrobot™ is used, changing the offset from 0mm to -1 or -2mm can also help.

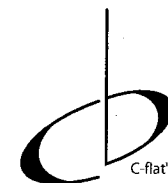
#### Hydrophilicity / Hydrophobicity

Increasing the hydrophilicity of the carbon film will help a droplet spread evenly over the carbon, rather than pool on the surface. The most common method for achieving this is by plasma or glow discharge; recommended settings for various equipment are given. Keep in mind that C-flat™ is manufactured without any plastics or soft materials in the process,

To reorder C-flat™ products or receive additional technical and product information, contact:

Protochips, Inc.  
[www.protochips.com](http://www.protochips.com)

Electron Microscopy Sciences  
[www.emsdiasum.com/microscopy](http://www.emsdiasum.com/microscopy)



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therefore plasma or glow discharge steps are only needed to make the surface more hydrophilic, not to clean. For this reason, a lower power and time is generally used.

#### Adding Carbon to C-flat™

Many C-flat™ parts are now offered in both the standard as well as a thicker carbon film, designed to give each lab the option to choose not only the most appropriate hole geometry and size, but also the ideal carbon thickness for their application. More information can be found on the Protochips, Inc. or EMS C-flat™ websites. In addition, users can add carbon to C-flat™ either to thicken the existing hole pattern, or as a thin continuous overlay across the hole pattern. Overlays are often used when particles have a strong affinity towards the carbon material.

#### Keeping the Carbon Intact

C-flat™ is designed to be an "out of the box" solution. Extensive sample preparation steps are generally not required, and often carbon that is torn or broken is a sign of plasma cleaning that is too long and/or at too high a power setting. Please refer to the suggestions on plasma cleaner settings, as well as on working with viscous samples.