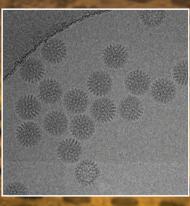


The premier holey carbon grid for cryo-transmission electron microscopy

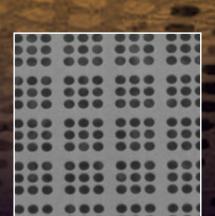


$\begin{array}{c|c} \textbf{C-f|c|}^{\top M} \\ \textbf{Holey Carbon Grids} \\ \textbf{for Cryo-TEM} \end{array}$





Electron Microscopy Sciences



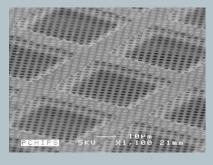


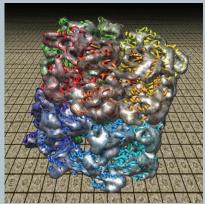
C-flat™ Holey Carbon Grids for Cryo-TEM

The premier holey carbon grid for cryo-transmission electron microscopy

Overview

C-flat™ is an ultra-flat, holey carbon-coated TEM support grid for transmission electron microscopy (TEM). Unlike competing holey carbon films, C-flat™ is manufactured without plastics, so it is clean upon arrival and the user has no residue to contend with.





250,000 particles of GroEL in 24 hours. Image Courtesy of Scott Stagg and Mike Pique NRAMM, The Scripps Research Institute (data acquired on CF-2/2-4C)

Articles

An improved holey carbon film for cryo-electron microscopy. Quispe J, Damiano J, Mick SE, Nackashi DP, Fellmann D, Ajero TG, Carragher B, Potter CS, (2007). Microscopy and microanalysis, 13(5), 365-371.

Improving the technique of vitreous cryo-sectioning for cryo-electron tomography: electrostatic charging for section attachment and implementation of an anti-contamination glove box. Pierson J, Fernández JJ, Bos E, Amini S, Gnaegi H, Vos M, Bel B, Adolfsen F, Carrascosa JL, Peters PJ, J Struct Biol. 2010 Feb;169(2): 219-25. Epub 2009 Oct 12.

C-flat™ is a trademark of Protochips, Inc. All rights reserved





The C-flat™ Advantage

C-flat™ leads to better data sets.

Made with patented technology, C-flat™ provides an ultra-flat surface that results in better particle dispersion and more uniform ice thickness.

Patterning is done using deep-UV projection lithography, ensuring the most accurate and consistent hole shapes and sizes down to submicron features. The precise methods by which C-flat™ is manufactured eliminate artifacts such as excess carbon and edges around holes.

C-flat™ is affordable

C-flat[™] is available in 25, 50, and 100 packs at a per-grid price less than competing products.

Applications

C-flat™ holey carbon grids provide the ideal specimen support to achieve high resolution data in cryo-TEM making them an ideal choice for single particle analysis, cryo electron tomography and automated TEM analysis.

Cryo-electron tomography (cryoET) and Single Particle Analysis (SPA):

Numerous researchers have reported that the ultraflat surface of C-flat™ leads to even ice thickness and uniform particle distribution within the hole areas. This optimal particle distribution results in superior data being collected as compared with other holey support films. 2µm hole sizes are standard but custom hole sizes are available so C-flat™ can accommodate the common magnifications used for quantitative TEM analysis.

Automated TEM:

C-flat™ provides a regular array of analysis sites compatible with automated data collection software such as Leginon. This compatibility, in combination with the more uniform ice thickness and particle distribution reported by numerous researchers, results in more high-quality target sites per grid.

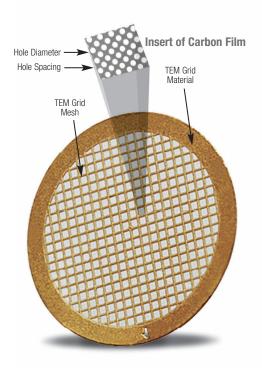
Product Line

C-flat™ is a holey carbon film supported by a standard TEM grid. C-flat™ products are fully specified by 4 parameters: the hole diameter and pitch of the holey carbon film and the material type and mesh size of the TEM grid. The image at top right illustrates these parameters.

Standard Products

The breadth of applications in cryoTEM necessitate a wide range of holey carbon film patterns. And now, with the recent expansion of the product line, a C-flat™ holey carbon film is available for almost any application. Whether 600nm holes are needed for very high magnifications with ultra-high resolution cameras or large open areas are needed for larger specimens, C-flat™ is the perfect holey carbon grid.

C-flat[™] is immediately available in several standard array patterns including hole diameters/hole spacings



of 0.6/2, 1/1, 1/2, 1/4, 1.2/1.3, 2/1, 2/2, 2/4, 4/2, and a multihole pattern. C-flat[™] is supported by your choice of a 200 mesh or 400 mesh copper TEM grid and sold in quantities of 25, 50, or 100.

Thick Products

C-Flat™ is available in a thick option that doubles the carbon thickness from approximately 20nm to 40nm. Thick C-flat product numbers end in -T, catalog numbers contain "CFT". Available in quantities of 50 and 100 per pack.

C-flat™ Customization

We realize that each customer has unique needs since specimens vary greatly in composition and size. To meet the diverse and demanding needs of the cryoTEM community, C-flat™ can be customized to meet a user's specific requirements. For example, C-flat[™] can be manufactured on other grid types such as 100x400 mesh grids, or London Finder grids. The size, shape and spacing of the holes perforating the carbon film can also be customized. For examples, those using electron tomography techniques might desire a larger hole size to allow for increased tilt angles; those using very high magnifications might find a smaller hole size desirable; 2D crystallographers might prefer a sparse hole pattern to take advantage of the clean and ultra flat surface of C-flat™; and based upon the specimen preparation and imaging protocols, grid metals other than copper might be required.

 $C ext{-flat}^{ ext{TM}}$ can be customized to meet all of these needs.

Please contact EMS with any custom C-flat[™] requests. We will be glad to provide you with a quote for specialized C-flat[™] grids. Requests for customized parts can be made directly to EMS via e-mail to s info@scienceservices.de.

C-flat[™] Holey Carbon Grids for TEM

Copper Only

Product Code	Cat. #	Hole Size	Hole Spacing	TEM Mesh	TEM Grid	Qty.
CF-1/1-2C	CF21-25	1.0 µm	1.0 µm	200	Cu	25/pk.
	CF21-50	1.0 µm	1.0 µm	200	Cu	50/pk.
	CF21-100	1.0 µm	1.0 µm	200	Cu	100/pk.
CF-1/1-2C	CF31-25	1.0 µm	1.0 µm	300	Cu	25/pk.
	CF31-50	1.0 µm	1.0 µm	300	Cu	50/pk.
	CF31-100	1.0 µm	1.0 µm	300	Cu	100/pk.
CF-1/1-4C	CF41-25	1.0 µm	1.0 µm	400	Cu	25/pk.
	CF41-50	1.0 µm	1.0 µm	400	Cu	50/pk.
	CF41-100	1.0 µm	1.0 µm	400	Cu	100/pk.
CF-1.2/1.3-2C	CF213-25	1.2 µm	1.3 µm	200	Cu	25/pk.
	CF213-50	1.2 µm	1.3 µm	200	Cu	50/pk.
	CF213-100	1.2 µm	1.3 µm	200	Cu	100/pk.
CF-1.2/1.3-3C	CF313-25	1.2 µm	1.3 µm	300	Cu	25/pk.
	CF313-50	1.2 µm	1.3 µm	300	Cu	50/pk.
	CF313-100	1.2 µm	1.3 µm	300	Cu	100/pk.
CF-1.2/1.3-4C	CF413-25	1.2 µm	1.3 µm	400	Cu	25/pk.
	CF413-50	1.2 µm	1.3 µm	400	Cu	50/pk.
	CF413-100	1.2 µm	1.3 µm	400	Cu	100/pk.
CF-2/0.5-2C	CF205-25	$2.0\;\mu\text{m}$	0.5 µm	200	Cu	25/pk
	CF205-50	2.0 µm	0.5 µm	200	Cu	50/pk
	CF205-100	2.0 µm	0.5 µm	200	Cu	100/pk
CF-2/0.5-3C	CF305-25	2.0 µm	0.5 µm	300	Cu	25/pk
	CF305-50	2.0 µm	0.5 µm	300	Cu	50/pk
	CF305-100	2.0 µm	0.5 µm	300	Cu	100/pk
CF-2/0.5-4C	CF405-25	2.0 µm	0.5 μm	400	Cu	25/pk
	CF405-50	2.0 µm	0.5 μm	400	Cu	50/pk
	CF405-100	2.0 µm	0.5 µm	400	Cu	100/pk
CF-2/1-2C	CF212-25	2.0 µm	1.0 µm	200	Cu	25/pk.
	CF212-50	2.0 µm	1.0 µm	200	Cu	50/pk.
	CF212-100	2.0 µm	1.0 µm	200	Cu	100/pk.
CF-2/1-3C	CF312-25	2.0 µm	1.0 µm	300	Cu	25/pk.
	CF312-50	2.0 µm	1.0 µm	300	Cu	50/pk.
05.0/4.40	CF312-100	2.0 μm	1.0 µm	300	Cu	100/pk.
CF-2/1-4C	CF412-25	2.0 µm	1.0 µm	400	Cu	25/pk.
	CF412-50	2.0 µm	1.0 µm	400	Cu	50/pk.
05.0/0.00	CF412-100	2.0 μm	1.0 µm	400	Cu	100/pk.
CF-2/2-2C	CF-222C-25	2.0 μm	2.0 µm	200	Cu	25/pk.
	CF-222C-50	2.0 µm	2.0 µm	200	Cu	50/pk.
CE 2/2 2C	CF-222C-100	2.0 µm	2.0 µm	200	Cu	100/pk.
CF-2/2-3C	CF-223C-25	2.0 µm	2.0 µm	300	Cu	25/pk.
	CF-223C-50	2.0 µm	2.0 µm	300	Cu	50/pk.
CF-2/2-4C	CF-223C-100 CF-224C-25	·	2.0 µm	300	Cu Cu	100/pk.
01-2/2-40	CF-224C-25	2.0 µm	2.0 µm	400	Cu	25/pk.
	CF-224C-50	2.0 µm	2.0 µm	400	Cu	50/pk. 100/pk.
	UF-224U-100	2.0 µm	2.0 µm	400	Gu	100/μκ.

C-flat™ Holey Carbon Grids for TEM (cont.) **Copper Only**

Product Code	Cat. #	Hole Size	Hole Spacing	TEM Mesh	TEM Grid	Qty.
CF-2/4-2C	CF242-25	2.0 µm	4.0 µm	200	Cu	25/pk.
	CF242-50	2.0 µm	4.0 µm	200	Cu	50/pk.
	CF242-100	2.0 µm	4.0 µm	200	Cu	100/pk.
CF-2/4-3C	CF342-25	2.0 µm	4.0 µm	300	Cu	25/pk.
	CF342-50	2.0 µm	4.0 µm	300	Cu	50/pk.
	CF342-100	2.0 µm	4.0 µm	300	Cu	100/pk.
CF-2/4-4C	CF442-25	2.0 µm	4.0 µm	400	Cu	25/pk.
	CF442-50	2.0 µm	4.0 µm	400	Cu	50/pk.
	CF442-100	2.0 µm	4.0 µm	400	Cu	100/pk.
CF-4/1-2C	CF241-25	4.0 µm	1.0 µm	200	Cu	25/pk
	CF241-50	4.0 µm	1.0 µm	200	Cu	50/pk
	CF241-100	4.0 µm	1.0 µm	200	Cu	100/pk
CF-4/1-2C	CF341-25	4.0 µm	1.0 µm	300	Cu	25/pk
	CF341-50	4.0 µm	1.0 µm	300	Cu	50/pk
	CF341-100	4.0 μm	1.0 µm	300	Cu	100/pk
CF-4/1-4C	CF441-25	4.0 µm	1.0 µm	400	Cu	25/pk
	CF441-50	4.0 µm	1.0 µm	400	Cu	50/pk
	CF441-100	4.0 µm	1.0 µm	400	Cu	100/pk
CF-4/2-2C	CF422-25	4.0 µm	2.0 µm	200	Cu	25/pk.
	CF422-50	4.0 µm	2.0 µm	200	Cu	50/pk.
	CF422-100	4.0 µm	2.0 µm	200	Cu	100/pk.
CF-4/2-2C	CF423-25	4.0 µm	2.0 µm	300	Cu	25/pk.
	CF423-50	4.0 µm	2.0 µm	300	Cu	50/pk.
	CF423-100	4.0 μm	2.0 µm	300	Cu	100/pk.
CF-4/2-4C	CF424-25	4.0 µm	2.0 µm	400	Cu	25/pk.
	CF424-50	4.0 μm	2.0 µm	400	Cu	50/pk.
	CF424-100	4.0 μm	2.0 µm	400	Cu	100/pk.
CF-MH-2C	CF2MH-25	Multihole*	_	200	Cu	25/pk.
	CF2MH-50	Multihole*	_	200	Cu	50/pk.
	CF2MH-100	Multihole*	_	200	Cu	100/pk.
CF-MH-2C	CF3MH-25	Multihole*	_	300	Cu	25/pk.
	CF3MH-50	Multihole*		300	Cu	50/pk.
	CF3MH-100	Multihole*	_	300	Cu	100/pk.
CF-MH-4C	CF4MH-25	Multihole*	_	400	Cu	25/pk.
	CF4MH-50	Multihole*	_	400	Cu	50/pk.
	CF4MH-100	Multihole*	_	400	Cu	100/pk

^{*} The Multihole device has a staggered pattern of six features consisting of three circle patterns of 1 micron, 1.4 micron and 2 micron diameter and three ellipse patterns of 1x4 microns, 1.4 x 5.6 microns and 2x8 microns.

C-flat™ Holey Thick Carbon Grids for TEM Copper Only

TEM Product Code Cat. # Qty. CF-1/1-2C-T CFT21-50 1.0 μm 1.0 μm 200 Cu 50/pk. CFT21-100 1.0 μm 1.0 μm 200 Cu 100/pk. CF-1/1-3C-T CFT31-50 1.0 μm 1.0 μm 300 Cu 50/pk. CFT31-100 1.0 μm 1.0 μm Cu 100/pk. 300 CF-1/1-4C-T 1.0 μm 1.0 μm CFT41-50 400 Cu 50/pk. 1.0 μm 1.0 μm CFT41-100 400 Cu 100/pk. CF-1.2/1.3-2C-T CFT213-50 1.2 μm 1.3 μm 50/pk. 200 Cu CFT213-100 1.2 μm 1.3 μm 200 Cu 100/pk. CF-1.2/1.3-3C-T **CFT313-50** 1.2 μm 1.3 μm 300 Cu 50/pk. CFT313-100 1.2 μm 1.3 μm 300 100/pk. Cu CF-1.2/1.3-4C-T **CFT413-50** 1.2 μm 1.3 μm 400 Cu 50/pk. CFT413-100 1.2 μm 1.3 μm 400 Cu 100/pk. CF-2/0.5-2C-T **CFT205-50** 2.0 μm 0.5 μm 200 Cu 50/pk CFT205-100 2.0 μm 0.5 μm 200 Cu 100/pk CF-2/0.5-3C-T **CFT305-50** 2.0 μm 0.5 μm 300 Cu 50/pk CFT305-100 2.0 μm 0.5 μm 300 100/pk Cu CF-2/0.5-4C-T **CFT405-50** 2.0 μm 0.5 μm 400 Cu 50/pk CFT405-100 2.0 μm 0.5 μm 400 Cu 100/pk CF-2/1-2C-T CFT212-50 2.0 μm 1.0 μm 200 Cu 50/pk. CFT212-100 2.0 μm 1.0 μm 200 Cu 100/pk. CF-2/1-3C-T CFT312-50 2.0 μm 1.0 μm 300 Cu 50/pk. CFT312-100 2.0 μm 1.0 μm Cu 100/pk. 300 CF-2/1-4C-T CFT412-50 2.0 μm 1.0 μm 400 Cu 50/pk. CFT412-100 2.0 µm 1.0 µm 400 100/pk. Cu CF-2/2-2C-T **CFT-222C-50** 2.0 μm 2.0 μm 200 Cu 50/pk. CFT-222C-100 2.0 μm 2.0 μm 200 Cu 100/pk. CF-2/2-3C-T **CFT-223C-50** 2.0 μm 2.0 μm 300 50/pk. Cu CFT-223C-100 2.0 μm 2.0 μm 300 Cu 100/pk. CF-2/2-4C-T CFT-224C-50 2.0 μm 2.0 μm 400 Cu 50/pk. CFT-224C-100 2.0 μm 2.0 μm 400 Cu 100/pk. CF-2/4-2C-T CFT242-50 2.0 μm 4.0 μm 200 Cu 50/pk. CFT242-100 2.0 μm 4.0 μm 200 Cu 100/pk. CF-2/4-3C-T CFT342-50 2.0 μm 4.0 μm 300 Cu 50/pk. CFT342-100 2.0 um 4.0 um 100/pk. 300 Cu CF-2/4-4C-T CFT442-50 2.0 μm 4.0 μm 400 50/pk. Cu CFT442-100 2.0 μm 4.0 μm 400 Cu 100/pk. CF-4/1-2C-T CFT241-50 4.0 μm 1.0 μm 200 Cu 50/pk CFT241-100 4.0 μm 1.0 μm 200 Cu 100/pk CF-4/1-3C-T CFT341-50 4.0 μm 1.0 μm 300 Cu 50/pk 100/pk CFT341-100 4.0 μm 1.0 μm 300 Cu CF-4/1-4C-T CFT441-50 4.0 μm 1.0 μm 400 Cu 50/pk CFT441-100 4.0 μm 1.0 μm 100/pk 400 Cu

C-flat™ Holey Thick Carbon Grids for TEM (cont.) Copper Only

Product Code	Cat. #	Hole Size	Hole Spacing	TEM Mesh	TEM Grid	Qty.	
CF-4/2-2C-T	CFT422-50	4.0 µm	2.0 µm	200	Cu	50/pk.	
	CFT422-100	4.0 µm	2.0 µm	200	Cu	100/pk.	
CF-4/2-3C-T	CFT423-50	4.0 µm	2.0 µm	300	Cu	50/pk.	
	CFT423-100	4.0 µm	2.0 µm	300	Cu	100/pk.	
CF-4/2-4C-T	CFT424-50	4.0 µm	2.0 µm	400	Cu	50/pk.	
	CFT424-100	4.0 µm	2.0 µm	400	Cu	100/pk.	
CF-MH-2C-T	CFT2MH-50	Mult	tihole*	200	Cu	50/pk.	
	CFT2MH-100	Mult	tihole*	200	Cu	100/pk.	
CF-MH-3C-T	CFT3MH-50	Mult	tihole*	300	Cu	50/pk.	
	CFT3MH-100	Mult	tihole*	300	Cu	100/pk.	
CF-MH-4C-T	CFT4MH-50	Mult	tihole*	400	Cu	50/pk.	
	CFT4MH-100	Mult	tihole*	400	Cu	100/pk	

C-flat™ Holey Carbon Grids for TEM Gold Only

Product Code	Cat. #	Hole Size	Hole Spacing	TEM Mesh	TEM Grid	Qty.
CF-1/1-2Au	CF21-100-Au	1.0 µm	1.0 µm	200	Au	100/pk.
CF-1/1-3Au	CF31-100-Au	1.0 µm	1.0 µm	300	Au	100/pk.
CF-1/1-4Au	CF41-100-Au	1.0 µm	1.0 µm	400	Au	100/pk.
CF-1.2/1.3-2Au	CF213-100-Au	1.2 µm	1.3 µm	200	Au	100/pk.
CF-1.2/1.3-3Au	CF313-100-Au	1.2 µm	1.3 µm	300	Au	100/pk.
CF-1.2/1.3-4Au	CF413-100-Au	1.2 µm	1.3 µm	400	Au	100/pk.
CF-2/0.5-2Au	CF205-100-Au	2.0 µm	0.5 µm	200	Au	100/pk.
CF-2/0.5-3Au	CF305-100-Au	2.0 µm	0.5 µm	300	Au	100/pk.
CF-2/0.5-4Au	CF405-100-Au	2.0 µm	0.5 µm	400	Au	100/pk.
CF-2/1-2Au	CF212-100-Au	2.0 µm	1.0 µm	200	Au	100/pk.
CF-2/1-3Au	CF312-100-Au	2.0 µm	1.0 µm	300	Au	100/pk.
CF-2/1-4Au	CF412-100-Au	2.0 µm	1.0 µm	400	Au	100/pk.
CF-2/2-2Au	CF222C-100-Au	2.0 µm	2.0 µm	200	Au	100/pk.
CF-2/2-3Au	CF223C-100-Au	2.0 µm	2.0 µm	300	Au	100/pk.
CF-2/2-4Au	CF224C-100-Au	2.0 µm	2.0 µm	400	Au	100/pk.
CF-2/4-2Au	CF242-100-Au	2.0 µm	4.0 µm	200	Au	100/pk.
CF-2/4-3Au	CF342-100-Au	2.0 µm	4.0 µm	300	Au	100/pk.
CF-2/4-4Au	CF442-100-Au	2.0 µm	4.0 µm	400	Au	100/pk.
CF-4/1-2Au	CF241-100-Au	4.0 µm	1.0 µm	200	Au	100/pk.
CF-4/1-3Au	CF341-100-Au	4.0 µm	1.0 µm	300	Au	100/pk.
CF-4/1-4Au	CF441-100-Au	4.0 µm	1.0 µm	400	Au	100/pk.
CF-4/2-2Au	CF422-100-Au	4.0 µm	2.0 µm	200	Au	100/pk.
CF-4/2-3Au	CF423-100-Au	4.0 µm	2.0 µm	300	Au	100/pk.
CF-4/2-4Au	CF424-100-Au	4.0 µm	2.0 µm	400	Au	100/pk.
CF-MH-2Au	CF2MH-100-Au	Multihole*		200	Au	100/pk.
CF-MH-3Au	CF3MH-100-Au	Multihole*		300	Au	100/pk.
CF-MH-4Au	CF4MH-100-Au	Mul	tihole*	400	Au	100/pk.

^{*} The Multihole device has a staggered pattern of six features consisting of three circle patterns of 1 micron, 1.4 micron and 2 micron diameter and three ellipse patterns of 1x4 microns, 1.4 x 5.6 microns and 2x8 microns.

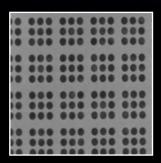
C-flat™

Holey Carbon Grids for Cryo-TEM

Frozen-hydrated Bacteriophage Capsid (data acquired on CF-1.2/1.4-4C).

Consistent

Researchers around the world have reported that the ultraflat surface of C-flat™ leads to even ice thickness and uniform particle distribution, allowing for superior 3-D reconstructions. 2 µm hole sizes are standard, but various hole sizes are available to accommodate different particle sizes and magnifications.



Compatible

C-flat™ provides a regular array of analysis sites compatible with automated data collection software such as Leginon. This compatibility, in combination with the more uniform ice thickness and particle distribution reported by numerous researchers, results in more high-quality target sites per grid.



Clean

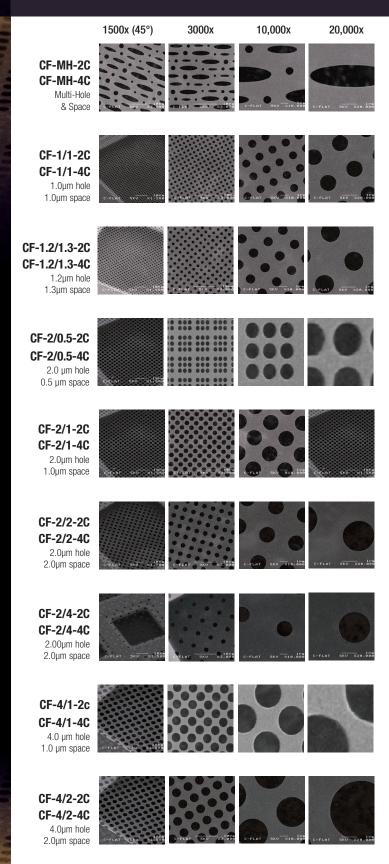
C-Flat™ uses no plastics or polymers in its production. This means C-Flat™ is shipped clean, so it's ready to use out of the box and requires no solvent washing steps prior to use, leading to less breakage of the holey carbon film.

Expanded Product Line

The breadth of applications in cryoTEM necessitate a wide range of holey carbon film patterns. And now, with the recent expansion of the product line, a C-flat holey carbon film is available for almost any application.

Standard Array Patterns

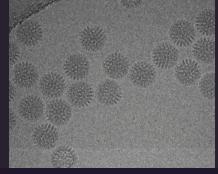
C-flat™ mounted on a stub using carbon tape and imaged with a Field Emission Scanning Electron Microscope



C-flat™

Holey Carbon Grids for Cryo-TEM

Cryo Preparation Using C-flat™



Frozen-Hydrated Bacteriophange Capsid (data acquired on CF-1.2/1.3-4C)

Overview

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.

Plasma Preparation

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

Note: 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.

Plunge Freezing

Recommended settings for plunge freezing with the $Vitrobot^{TM}$

• Temperature: 4°C

• **Humidity:** 100% (can vary between 90–100%)

• Blot Time: 3-5 seconds

• Volume on Grid: 3µL (can vary)

• **Drain Time:** 0 seconds

• Offset: 0 for regular samples, -1 for viscous

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.

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 1µL 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 2.0µL 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 VitrobotTM 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, 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. In addition, carbon can be added 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.

Publications using C-flat™:

Near-atomic resolution using electron cryomicroscopy and single-particle reconstruction. Proceedings of the National Academy of Sciences, Volume 105, Number 6, pp. 1867-1872, 2008. X. Zhang, E. Settembre, C. Xu, P. R. Dormitzer, R. Bellamy, S. C. Harrison, and N. Grigorieff

Preparation of macromolecular complexes for cryo-electron microscopy. Nature Protocols, Volume 2, pp. 3239 - 3246, 2007. R. A. Grassucci, D. J. Taylor, and J. Frank

Segrosome structure revealed by a complex of ParR with centromere DNA. Nature, Volume 450, pp. 1268-1271, 2007. M. A. Schumacher, T. C. Glover, A. J. Brzoska, S. O. Jensen, T. D. Dunham, R. A. Skurray and N. Firth

Automation of random canonical tilt and orthogonal tilt data collection using feature-based correlation. Journal of Structural Biology, Volume 159, Issue 3, pp. 335-346, September 2007. C. Yoshioka, J. Pulokas, D. Fellmann, C. S. Potter, R. A. Milligan and B. Carragher

Automated cryoEM data acquisition and analysis of 284 742 particles of GroEL. Journal of Structural Biology, Volume 155, Issue 3, pp. 470-481, September 2006. S. M. Stagg, G. C. Lander, J. Pulokas, D.s Fellmann, A. Cheng, J. D. Quispe, S. P. Mallick, R. M. Avila, B. Carragher and C. S. Potter

Contamination buildup limit throughput for automated cryoEM? Journal of Structural Biology, Volume 154, Issue 3, pp. 303-311, June 2006. A. Cheng, D. Fellmann, J. Pulokas. C. S. Potter and B. Carranher



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