

C-Dish



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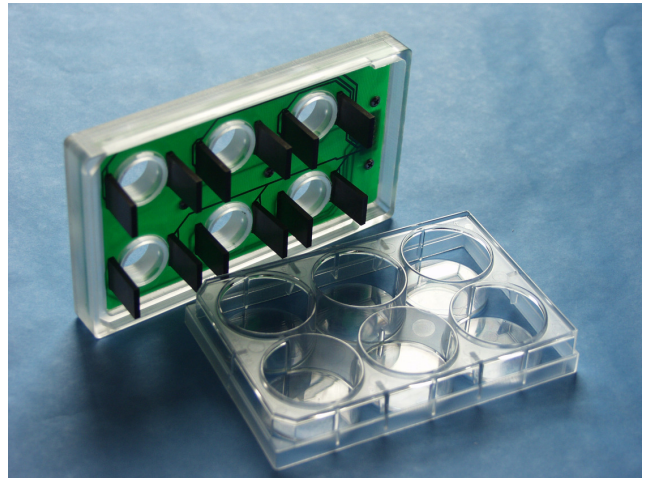
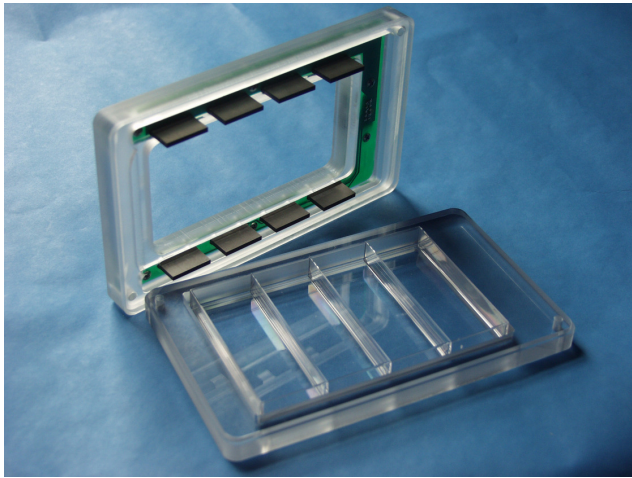
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Introduction



The patented C-Dish works in conjunction with the C-Pace and C-Pace EP bulk cell stimulators to form a complete system for chronic stimulation of bulk quantities of cardiac myocytes (and other excitable cells) in culture. The C-Dish is a simple assembly that precisely places carbon electrode elements in a standard culture dish. A ribbon cable connector provides access to the field stimulating electrodes. Varieties of C-Dishes are available to work with 4, 6, and 8 well dishes as well as 35 mm dishes and the Flexcell UniFlex.

Features

- Physically stable assembly with standard culture dishes
- Carbon electrodes effectively stimulate cells without releasing toxic electrolysis byproducts into media

Inputs / outputs

Standard ribbon cable connector

Description of C-Dish

The IonOptix C-Pace and C-Pace EP culture pacing systems are designed to enable chronic electrostimulation (“pacing”) of cells in culture. The C-Dish electrode assemblies are composed of one or two Lexan polycarbonate shells. The Lexan housing forms the structural support to hold a circuit board with protruding carbon stimulation electrodes. The carbon electrodes are soldered to the underside of the board via gold-plated pins inserted into the electrodes, providing mechanical and electrical contact. The electrodes protrude into the well of the tissue culture dish and sit immersed in the culture media. A 10-pin connector is soldered to the top of the circuit board. The connector’s plastic housing and pins protrude through the polycarbonate on the top of the board, allowing voltage to pass from the stimulator’s high voltage boards to the C-Dish via a ribbon cable that can be passed between the tissue culture incubator’s gasketed door and the incubator itself. When the ribbon cable is plugged in, the electrodes are electrically connected to reed relays within the culture pacer. As only one relay pair is closed at a time, current flow is guaranteed to be zero between pulses. The C-Pace will close the relay pairs one at a time, so the wells are paced sequentially. Sylgard (a polydimethylsiloxane elastomer) is used to seal the C-Dish circuit board.

Additional Required Components

IonOptix C-Pace EP:

The C-Pace EP is a multi-bank cell culture stimulator, available from IonOptix. The high voltage section of the C-Pace EP consists of up to 8 independent banks with multiple channels each. The EHV100 banks have a dip switch which can be changed to allow it to work with any dish. One high voltage bank will pace a single C-Dish. Identical pulses are sent sequentially through the channels of a bank. The high voltage amp is connected to each channel through its own reed relay, to ensure a completely open circuit between pulses. The amplifier provides an output of up to +/-40 V and up to 240 mA. This output has been shown experimentally to be sufficient for the standard wells we support. The C-Pace EP is connected to the stimulation electrodes via a 9 connector ribbon cable. The cable is thin enough to close the incubator door on and maintain a good seal.

Disposable Culture Dishes:

We support several different varieties of sterilized, disposable dishes that are available through Fisher, VWR and a number of other sources. More information is available at <http://www.nuncbrand.com/>, www.bdbiosciences.com/, <https://www1.fishersci.com/>, and <http://www.flexcellint.com/>. See Appendix A for a list of known part numbers.

IonOptix C-Dish Part Number	Compatible Disposable Dish Information (IonOptix dishes have been tested for fit with listed part number, but should fit most variations.)
CLD4WN	Nunclon Δ Polystyrene Rectangular Well Dishes: 4 well dish (Nalge Nunc International #167063) are widely available. They hold four 24 x 50 mm cover slips.
CLD8WN	Nunclon Δ Polystyrene Rectangular Well Dishes: 8-well dish (Nalge Nunc International #167064) are widely available. They hold eight 22x22 mm cover slips.
CLD6W35CN	Fits Nunclon and Corning 35 x 10mm dish
CLD6W35F	Fits Falcon Brand 35 x 10mm dishes
CLD6WFC	BD BioCoat #353846 or Corning #3516 Multiwell Plates: 6-well 35 mm
CLD6WFLEX	UniFlex Culture Plates: 6-well (Flexcell)
CLD12WFC	Fits Falcon Brand 12-well plates
CLD24WF	Fits Falcon Brand 24-well plates

Assembly

6-well dishes have large stable bottom plates, so the compatible IonOptix C-Dish only consists of a top plate. The rest have bottom plates for more stability. To facilitate assembly, drop the tissue culture plate bottom into the bottom part of the C-Dish before cells and media are added. This part of the assembly can stay together for the duration of the culturing period. Add the cells and media. Fit the C-Dish top onto the assembly so the C-Dish electrodes descend into the media. Use thumbscrews to hold top and bottom plates together. The tissue culture plate lid will fit over the groove on top. For changing media, only this lid will need to be removed. To remove the electrodes for cleaning, remove the thumbscrews and replace the entire top half of the C-Dish with a new one. To connect to the C-Pace, plug the ribbon cable in to the ribbon cable connector. It is polarized, so it will only fit in the correct way. Run the ribbon cable through the door opening of the incubator. The door will close tightly around it to maintain a good seal.

Cleaning

The carbon electrodes absorb salts, electrolysis byproducts and protein from the media, and will need to be cleaned every 24-48 hours. Toxic chemicals leached from the electrodes into the media will kill cells in culture if the electrodes aren't cleaned regularly. The normal method is simple soaking in distilled water with a stirrer for a couple of days. The water should be changed occasionally until the distilled water stops turning pink from residual pH indicator (phenol red) leaching from the carbon. The whole top dish assembly can be soaked. The white plug the dishes are shipped with is intended to provide protection for the 10 pin connector while cleaning.

We do not recommend the use of an autoclave for sterilization. If the C-Dish must be completely sterilized, they should be thoroughly rinsed first, and the autoclaving cycles should be limited to 20 minutes at 121°C. Do not use strong alkaline detergents. Autoclaving poses a considerable stress on the dishes. The Lexan polycarbonate used to provide support for the C-Dish has a softening temperature of 145°C (the temperature at which the dishes will warp). While this is well above the temperatures typically used in an autoclave, the severe geometry of the CDishes may result in a slightly lower softening temperature. Autoclaves can be poorly calibrated as well, increasing the risk of doing irreparable damage to the assemblies. Additionally, the conductive epoxy maintaining electrical connectivity between the electrical boards and the carbon electrodes may be damaged. Many C-Pace users prefer to disinfect their C-Dishes with 70% EtOH as an alternative to sterilization in an autoclave. This disinfection step can be followed by leaving the dishes with the carbon electrodes up under the UV germicidal lamp in the tissue culture hood. This will prolong the life of the C-Dish and should be sufficient to suppress microbial growth in combination with antibiotics.

It is also a good idea to buy a soft toothbrush and scrub the surface of the electrodes occasionally, especially if you start to notice a difference in the responsiveness or health of the cells. The electrodes are solid carbon, so don't be concerned about rubbing the surface off. The toothbrush will be pretty black when you are done.

Example Protocol

This protocol was provided by Dr. Joseph Cheung. Please keep in mind that he uses 4-well C-Dishes. Higher density multiwall dishes should not require 40 volts.

First, make sure that the carbon electrodes are squeaky clean. If they are not clean, your myocytes will surely die. After each use, we soak the electrodes in a beaker (1L) of distilled water, with a stir bar stirring for 6-12h, then change the water. We repeat the soaking/stirring procedure 3-4 times until the water is absolutely clear. You will be surprised how much of the culture media salts will leach out of the electrodes (as indicated by the water turning pink). We then autoclave the electrodes before use (*IonOptix note: we do not recommend placing your C-Dish in an autoclave*).

Second, we use 1Hz stimulation frequency at 40 volts (*IonOptix note: this protocol is designed around a 4-well assembly; 40 volts is too high for denser multiwell plates*).

Third, make sure your myocytes are stuck to your culture dish or coverslip. We precoat our dishes/coverglass with laminin before seeding the myocytes. We then let the myocytes sit (without stimulation) for 2-3h. Then we change the media (we use Media 199), let the myocytes sit (without stimulation) for another 3h before stimulation. Thus the myocytes will not see the electric current until 6h after isolation.

Fourth, pay attention to your cell isolation apparatus. Our Langendorff perfusion columns that we use to isolate myocytes are thoroughly rinsed with distilled water, followed by 70% ETOH, and then covered with tin-foil after each use. We autoclave our surgical instruments before each use. The cleaner your myocyte prep is, the easier it is to culture and pace them. Your culture media should be clear the next day and there should not be visible bacteria in the dishes.

Fifth, we change our culture media daily. We have been able to pace our myocytes for 6-7 days (for Western blots). We usually use ours after 2-3 days of pacing culture for contraction, patch-clamp, and fura-2 measurements. Unlike myocytes which are quiescent (not paced) in culture in which their contractility deteriorates rapidly within 6-18h, we found no deterioration in maximal contractile amplitudes even after 3 days of culture when compared to freshly isolated cells.

Appendix A

IonOptix C-Dish	Dish Brand	Known Part Numbers
CLD4WN	Nunclon 4-well Rectangular Dishes or Multidishes	267061, 167063
CLD6W35CN ¹	Nunclon 35mm Dishes	150318, 153066, 174926, 171099
	Corning 35mm Culture Dish	430588, 3294
	Mattek 35 mm Glass Bottom Dishes	Any of the series P35G*C or P35G*N (FYI- The *C series, especially the P35GC1514C, is readily available. MatTek only custom makes the *N series.)
CLD6W35F ¹	BD Falcon 35mm or 35x10 mm Dishes	354456, 356456, 354459, 354457, 354458, 354460, 356467, 354467, 354077, 356518, 354518, 354602, 351008, 353801, 353001
	Mattek 35 mm Glass Bottom Dishes	Any of the series P35G*F (FYI- MatTek only custom makes this series.)
CLD6WFC	BD Falcon 6-Well Plate	351146, 353934, 353046, 353224, 353224, 354431, 354417, 354671, 354721, 356721, 356773, 354773, 353846, 356400, 354400, 354428, 354402, 356652, 354652, 354404, 354510, 354432, 354603, 356413, 354413, 354595, 356515, 354515, 354658
	Corning 6-Well Plate	3335, 3471, 3506, 3516
CLD6WFLEX	FlexCell BioFlex and UniFlex Plates	BF-3001* series and UF-4001*series (FYI- there is currently no method of synchronizing the electrical and mechanical stimulation)
CLD8WN	Nunclon 8-well Rectangular Dishes or Multidishes	267062, 167064
CLD12WF	BD Falcon 12-Well Plate	353043, 353225, 351143
CLD24WF	BD Falcon 24-Well Plate	353226, 351147, 353047

¹ one of our CLD6W35* dishes holds six 35 mm dishes