WARNER INSTRUMENTS
A Harvard Apparatus Company

SF-77C Perfusion Fast-Step

SE Smart Ephys
a Harvard Bioscience, Inc. brand

The **SF-77C** is a simple and highly effective stimulus solution delivery device for use in a variety of patch clamp and electrophysiology studies. Control and test solutions flow continuously through adjacent solution delivery tubes and a stepper mechanism selects which tube is directed at the preparation. The rapid response and nominal hysteresis of the stepper mechanism allows for very fast switching times with complete solution changes typically achieved within 20 ms for a large 700 mm step. Times are significantly shortened as the step size is decreased.

Features of the **SF-77C** include:

- Solution stimulus delivery for patch clamp and other electrophysiology studies
- Solution changes in milliseconds (ms)
- Minimal flow turbulence
- No switching through intervening solutions
- Manual or automatic step control (digital or analog)
- Modest cost and easy maintenance
- Use with most chambers or culture dishes

THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED
FOR USE ON HUMAN SUBJECTS

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INTRODUCTION

Multiple solution studies

In the standard configuration, up to six different solutions are connected to a single input manifold which in turn is connected to one of three square glass stimulus ports. The three stimulus port design is superior to a two-port design in that complex solution exchange protocols can be brought to bear on the sample under study. Since the complete system is designed to accommodate three manifolds (one for each stimulus port) and each manifold can accommodate up to 6 feed lines, it is possible to immediately select between 18 different input solutions.

- Solution changes between stimulus ports occur within milliseconds.
- Changes between solutions connected to an individual port occur within five seconds.
- Entirely new solutions can be added into any port with a waiting time of no more than 30 seconds.
- The cell is never required to pass through intervening solutions to get from control to test solution.

Manual or External control

The stepper mechanism can be manually controlled via the front panel or externally directed from your data acquisition program. Manually, the system can be stepped to 8 positions in 7 equally spaced steps. These same 8 positions can also be directly selected by applying an analog signal to the external analog input BNC or by passing a 3 bit word to the TTL input on the instrument rear panel.

Square glass stimulus ports

The square glass tubes used for solution delivery significantly reduces mixing turbulence between solutions allowing the **SF-77C** to be used for studies with both membrane patches and whole cells, even when the cells are not fixed to a substrate.

System versatility

The design of the **SF-77C** permits the use of various size glass tubing for perfusion delivery.

SF-77C – Standard system (0.7 mm ID tubes)

The standard system is shipped with 3SG700-5 single-walled 3-barrel glass tubing which eliminates the need to glue individual barrels together. Spacing between barrels is 0.7 mm and step speed between adjacent barrels is typically 20 msec. Single barrel SG800-5 tubes (up to 5) can be used with the same holder.

SF-77CLT – Large tube system (1.0 mm ID tubes)

Larger stimulus ports are required when using the **SF-77C** with larger cell structures such as the *Xenopus* oocyte. Solutions are delivered through 1.0 mm ID square tubes (SG1000-5) with barrel-to-barrel spacing of 1.4 mm.

SF-77CST – 1 ms stepping with Theta tubing

The **SF-77C** has been successfully tested using theta style capillary tubing with a step speed of 1 ms between adjacent barrels. Standard 2 mm diameter theta tubing is pulled to a tip diameter of 300 mm with a spacing of 100 mm between barrels. In this design, placement of the pipette tip is critical due to the smaller perfusion stream. Since mechanical artifacts can be evident when using these small step sizes the motor drive voltage is adjustable at the rear panel.

Easy set-up

The stepper mechanism is compact, lightweight, and free of either mechanical or electrical noise. The mechanism connects to the control box with a 2 meter shielded cable and is provided with a mounting rod for attachment to a manipulator. Manifolds can support 2, 4 or 6 inputs depending on the experiment. Solutions flow from reservoirs to the manifold through PE-50 tubing and PE-10 tubing is used to connect the manifold outputs to the glass tubes.

HARDWARE DESCRIPTION

SF-77C Front Panel Controls

Mode select toggle switch

Control of the stepper device can be set to *INTERNAL* (manual), *EXT DIGITAL* or *EXT ANALOG*. The 3 position toggle is used to select the desired mode.

External control analog select

An analog signal for stepper control can be applied to a rear panel BNC input. Up to 8 positions can be selected. See rear panel description for controlling the **SF-77C** with an analog signals.

External control digital select

A digital signal for stepper control can be applied to three rear panel BNC inputs. Up to 8 positions can be selected. See rear panel description for controlling the **SF-77C** with digital signals.

Internal control (manual) position select

An 8-position selector is used to manually select up to 8 positions. The LED display indicates the position selected. This same display indicates the position selected when control is applied from one of the external sources.

Initialize

The INITIALIZE push-button is used to initialize the pipe position at the beginning of an experiment or at any time one wishes to check or confirm positions. This is done in the INTERNAL (manual) mode by selecting the desired position and depressing the push-button.

Steps/position pushbutton control

Step length (distance between stepper positions) is selected with the STEPS/POSITION pushbutton control. The minimum step length is 100 µm (0.004 inches) with the display set at 1, and the maximum step length is 1.5 mm (0.060 inches) with the display at 15.

When using the standard 3 barrel glass supplied with the **SF-77C** (3GC700-5, barrel to barrel spacing of 700 µm) in an unpulled configuration, the STEPS/POSITION control is set to 7 (7 x 100 µm).

SF-77C Rear Panel Inputs

DC power input

The external +12 V DC power supply cord connects here.

Motor

The stepper mechanism connects to the control box here.

Motor voltage adjust

This control is factory set and is only used for experiments involving small (100 µm) steps. Its use is described in the section *Fast stepping with Theta tubing.*

External digital input BNC's

Digital inputs for position control connect to the 3 BNC connectors labeled *D0*, *D1,* and *D2*. Up to 8 positions can be controlled when all 3 lines are used. Digital inputs are TTL compatible (low = 0 V; high= 5 V) and readily accept digital outputs from computers and computer interfaces. Positions are set per the truth table shown below.

EXTERNAL DIGITAL INPUT TRUTH TABLE			
Position	D0	D1	D2
0	L	L	L
1	H	L	L
2	L	H	L
3	H	H	L
4	L	L	H
5	H	L	H
6	L	H	H
7	H	H	H

External analog input BNC

Analog inputs for position control connect to the labeled BNC connector. Up to 8 positions can be controlled when the appropriate voltage is applied. Positions correspond to the DC voltage levels as shown below.

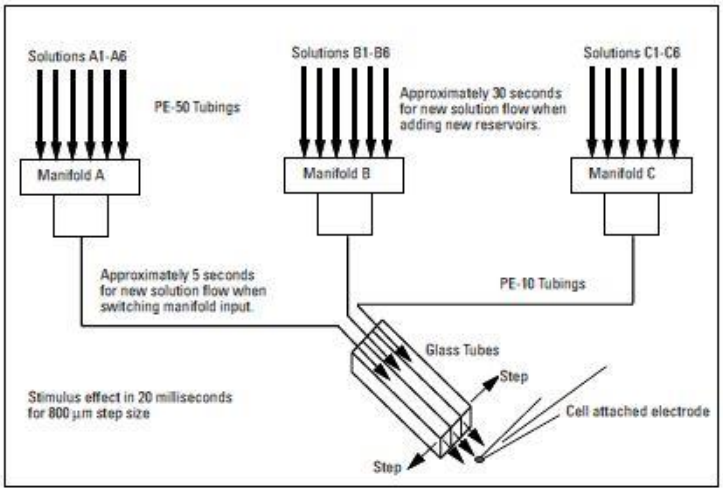
Step position	Input voltage		Step position	Input voltage
Position 0	0 volts		Position 4	4 volts
Position 1	1 volt		Position 5	5 volts
Position 2	2 volts		Position 6	6 volts
Position 3	3 volts		Position 7	7 volts

System Use

The main use of the **SF-77C** Perfusion Fast-Step is the rapid change of solutions bathing a single cell or patch of membrane attached to a patch electrode. Solution changes can be made in as little as 20-50 ms (approximately 1 ms using 2 barrel Theta tubing) and once changed, the new solution with its particular concentration of pharmacological agent will remain stable until changed again.

An important capability of this device is that a large number of solutions can be utilized during the course of a single experiment despite the compact size of the device. This can take a bit of organizational thought before starting the experiment, but the return on the effort is the ability to easily use 15-30 different solutions in the course of a single experiment.

This broad capability is accomplished by having as up to six solutions feeding through manifolds and into each of the three "pipes" in front of which the cell may be placed. This allows any of the three pipes to have any one of 6 solutions flowing through it at any time. It's even possible, during the course of an experiment, to change the six solutions flowing into any one of the manifolds, allowing a virtually limitless number of solution combinations to be used.



In order to fully implement this feature it's important to note that there are actually three time frames to consider for the proper use of the device.

The first is the time for shifting between output tubes (50 ms or less). The second is a 5 second dead time required to change one solution to another within the same output tube. Finally, there's a 30-60 second delay required to deliver one of the six *starting* solutions flowing into any of the three manifolds. This third time frame is only important for experiments that typically use more than 15 different solutions in a single experiment. Each of these uses will be explained in detail, and a "typical" experiment will be worked through using each of these features.

SET-UP PROCEDURE

The **SF-77C** systems are packaged in three parts:

1) Stepper motor assembly with a set of 3 manifolds*
2) Electronic controller
3) Glass holder, capillary tubes, and polyethylene tubing

* The **SF-77C** and **SF-77CST** are supplied with two 6-to-1 MM Series manifolds and one 2-to-1 MM Series manifold. The **SF-77CLT** is supplied with two 6-to-1 ML Series manifolds and one 2-to-1 ML Series manifold.

Capillary tubes and holder

Glass capillaries

The **SF-77C** makes use of square capillary tubes for its output tubes or "pipes". These facilitate placement of the tubes in the chamber and, more importantly, reduce turbulence and provide a more even flow of solution. The capillaries supplied with the standard **SF-77C** are 3-barrel which have an inside dimension of 0.6 mm each barrel and a wall of 0.1 mm. (Adjoining tubes share a single septum which determines a 0.7 mm center-to-center working distance.) The barrels have been fused together in manufacture so no gluing is necessary.

Larger 1.0 mm tubing is supplied with system **SF-77CLT**, and 2-barrel Theta tubing is supplied with the **SF-77CST**.

Glass holders

The appropriate glass holder is supplied with each system. The standard SF-77C holder is the **GH-1**. It holds the 3SG700-5 three-barrel tubing or up to 5 individual barrels of SG800-5 tubing (0.6 mm inside, 0.12 mm wall).

The SF-77CLT is supplied with the **GH-10** holder which holds up to 3 of the SG1000-5 glass tubes (1.0 mm inside, 0.2 mm wall).

The **GH-2T** holder is designed to hold 2 mm Theta glass.

Mounting the glass

The capillaries can be mounted directly in the holder, which maintains them in position by pressure. The 3-barrel glass is simply placed into the holder slot and the threaded handle is tightened to hold the glass rigid. If single tubes are used, we recommend that the capillaries be cemented together first to insure that they are perfectly level and aligned. Place the capillaries on a flat surface and butt the ends against a straight edge.

Proper alignment is critical. We suggest you check under a dissecting microscope. Use only a small dab of cement placed near, but not exactly at the center line in the lengthwise direction. Do not put the cement near either end of the tubes as capillary action will carry it into the tubes and may clog them.

After the cement dries (a few minutes is sufficient) simply slide the tubes into the holder such that nearly half of the tube length extends from the holder. This length is adjustable depending on the dimensions of your particular set-up and chamber. Ideally, however, it is best to have the tubes more or less centered on the holder.

The tubes and holder can be easily attached to the mounting device, but this should be done after it is installed as described in the next section.

Manifolds

Supplied manifolds contain 2 or 6 inputs. MM Series manifolds are standard with the **SF-77C** and **SF-77CST**. ML Series manifolds are provided with the **SF-77CLT**.

MM Series manifolds

The manifold inputs connect to PE-50 tubing which fits over the hypodermic tubing. A length of PE-10 tubing is factory installed in the manifold output. The output tubing from the fits directly into the glass **SF-77C** pipes.

The manifold PE-10 tubing will occasionally require replacement. Proceed as follows:

1) Unscrew the manifold tail from the head and pull the old tubing from the tail.
2) Cut a length of PE-10 tubing, 8 to 9 inches long.

3) You need to stretch one end of the tubing so that its diameter is reduced sufficiently to fit into the hole in the tail piece. Do this by gripping one end between thumb and forefinger of both hands and pulling firmly. This may take a little practice.
4) Trim off the un-stretched portion of tubing at the end so that the smaller diameter portion can be inserted into the hole. Pull the tubing through until the unstretched diameter is captured inside the tail. Trim the tubing flush with the tail inside end and reinstall in the head piece. Make sure the rubber seal is inside the head before screwing the parts together.

ML Series manifolds

These manifolds use the PE-50 tubing on both input and output ends. The larger PE-50 fits nicely into the SG1000-5 glass pipes used with the **SF-77CLT**.

Mounting the motor assembly

The **SF-77C** is designed to be mounted on and positioned by a manipulator. While it's light enough to be attached to a precision micromanipulator without producing drift. Fortunately, most applications only require a standard, coarse manipulator, which is more than sufficient for the task. Ideally, the **SF-77C** stepper assembly should be positioned within the set-up so that it can be easily removed from the manipulator and replaced. This will facilitate cleaning and loading the device.

Positioning the angle of the motor assembly is made by loosening the black wing nut. When loosened, the motor mount bracket and manifold bar can be rotated to accommodate the setup. Additionally, the angle of the motor extension bar (white plastic bar attached to the motor drive) can be adjusted by rotating the motor collar (black block mounted on the motor).

Before rotating the block, loosen the small set screw with the Allen wrench supplied with the **SF-77C** motor assembly. Re-tighten the screw lightly once the desired position is attained.

NOTE: Do not overtighten this screw as motor operation will be adversely effected.

A subsection of this part of the device is the assembly of tubing etc. that will feed solutions into the manifolds and then to the pipes. Most simply, the feeder arrangement consists of up to 18 syringes with 2-way Luer stopcocks. (3-way stopcocks can be used, but this tends to introduce air bubbles at the point of entry into the PE-50 tubing.) Be careful to avoid air bubbles, as once

in the PE-tubing, the flow of solution is inhibited. Depending on the rate of flow used for a particular application, this may not be detected immediately. Avoidance and removal of bubbles will be discussed later.

The syringes are mounted in a rack, loading end up, and placed approximately 1 meter above the surface on which the chamber is fastened (usually the microscope stage). The height above the device will depend on the experimental design, for example, perfusing an inside out patch will require a slower flow rate than perfusing a cell. The syringes can be mounted either inside or outside of the Faraday cage if one is being used. A distinct advantage to this system is that electrical noise is not a problem, even for single channel recording.

Syringes of 20 or 60 ml volume are recommended, although other containers such as separator funnels or bottles can be used. The main advantage of syringes is their economy and disposability. For expensive reagents smaller syringes can be used. With careful preparation of the solution feeder lines, the **SF-77C** can be reliably used with less than 1 ml of solution in the reservoir and with less than 0.5 ml to prime the line (depending on distance of solution reservoir from the microscope stage).

Loading solutions

It is recommended that one of the solutions in each of the three pipes should be the control buffer bathing the cells or patches. This solution can be used as a rinsing solution between applications. Solutions are poured into the syringes with the Luer stopcock set to the closed position.

A useful method for the removal of air bubbles is as follows. Remove the tip of a cotton tipped applicator and pack into the needle, this acts as a bubble trap. Even with careful removal of air bubbles prior to the solution reaching the PE-tubing, occasionally small bubbles will form. The cotton tip breaks up the bubble, then the smaller ones formed can be easily tapped out.

To prevent bubble formation in the bulk of the solution, always pre-warm solutions. This entire procedure is made easier by removing the manifold from the holding bar and unscrewing the tail portion of the manifold (the section of the manifold through which solutions flow out of the manifold). Because the output tubing is much smaller than the input tubes, the resistance is large enough to cause solution to back up into neighboring tubes during the filling procedure.

Removal of the output section of the manifold prevents this from occurring. After all the lines are filled, the manifold can be screwed together again and mounted back into the holder.

Next, remove the needle from the syringe. Hold the needle about 1-2 mm below the syringe, then open the Luer valve. Solution will flow under the force of gravity without the requirement for additional force. As soon as the solution reaches the end of the syringe, allow a small drop of solution to enter the needle. When the cotton is wet, quickly insert the needle onto the syringe tip. Observe the solution passing along the PE-50 tubing, and after making sure that there is no air in the tubing, switch the Luer valve to *closed*. Inspect the needle to see if any air bubbles are present and tap the needle to dislodge.

Often large bubbles will form at the junction of the syringe and the Luer valve, these can be easily removed by tapping. If this will not suffice, then insert a long needle into the solution and draw out the air bubble (care must be taken not to cross contaminate). If at this point even a small bubble remains, tap the syringe and dislodge. This can be rather time consuming, depending on the number of solutions to be used, but is a very useful exercise that will maximize the performance of the **SF-77** Perfusion Fast - Step.

Next attach the PE-50 tubing to the manifold. Open the Luer valve and allow solution to flow through the glass 'pipes'. If at this point there is inhibition of solution flow into the glass 'pipe', turn the Luer valve to closed and remove the outflowing section of the manifold. Insert into a piece of Tygon tubing (I.D. 1/16; O.D. 1/8; Wall 1/32) attached to a syringe, and simply aspirate and eject air. Re-attach the manifold and open the Luer valve. If there is no solution flow at this point apply positive pressure to the reservoir syringe (making sure all other feeder lines are closed otherwise back-flow will occur. The Luer valve should be briefly re-opened to be sure that solution is indeed flowing through the line. The same procedure is repeated for each solution in turn. After all six solutions have been readied in this way it is advisable to turn on the buffer solution line so as to clean the capillary pipe of any residual solutions. The same procedure should be repeated for each of the three capillary pipes. When completed all the solutions to be used in the experiment are at the manifold so that simply turning a stopcock to the on position will allow that solution to flow from the manifold to the capillary tube. This should require less than 2 seconds, but 5 seconds are recommended to be sure that the last solutions have been completely washed out. Although in general there is no leakage from the end of the pipes we recommend that at the start of an experiment all three pipes have buffer flowing through them (see sample Experiment below).

If a line is not to be used (i.e. if less than six solutions are to be used in any one pipe) then it is necessary to use a "plug" for that line on the manifold. This is to prevent solution from "backing up" into an open line. Alternatively all unused lines can simply be loaded with buffer solution, however this is more time consuming.

NOTE: It is best to keep the output ends of the pipes submerged in solution or water when changing the setup. If the pipes are left unsubmerged for any length of time, fluid will weep from the tubing and air bubbles will creep in.

Care and Maintenance

At the end of each experiment, allow all solutions to flow out of the reservoir and add a few ml of 70% ethanol. The manifold is composed of Delrin and stainless steel and PE-tubing is unaffected by ethanol, thus this cleaning procedure can be withstood. Next flush all the lines with water. After all solutions have run through, remove the needle and dispose of the cotton tip (prevent cross contamination of solutions). Finally air dry (pressurized) solution lines.

USING THE CONTROLLER

Control of the **SF-77C** has been designed to be as simple as possible. The device can be manipulated manually or by electronic signals generated by a computer or stimulator. The three main parameters under the investigators control are *position, step size* and *duration*.

Position

Position tells the device which capillary pipe to position in front of the target cell or membrane patch. There are 8 positions available in INTERNAL (manual) mode, and 8 positions available in the EXT ANALOG and EXT DIGITAL modes. The typical use is with 3 pipes and only three positions are required. Position is selected by manual switch position or by an external analog or digital signal. The position selected is displayed on the LED readout.

Analog Voltage Control

When using an analog input, the positions are voltage sensitive. Zero volts corresponds to 0 position and stepping the voltage in 1 volt increments steps the device.

Digital Control

The rear panel has 3 BNC inputs to accept standard TTL compatible signals for digital control from a computer or other digital signal source. The 3 inputs allow for as many as 8 positions to be controlled. See REAR PANEL DESCRIPTION for more detail.

Step size

Step size, adjustable from 0.1 - 1.5 mm in 0.1 mm steps, is selected for the center to center distance of the pipes.

The distance traveled is adjusted with the STEPS/POSITION counter control. The minimum step is 100 um or 0.1 mm (1 step) and the maximum is 1.5 mm (15 steps). For the standard 3SG700-5 capillary pipes supplied (barrel to barrel spacing 0.7 mm), a step length of 7 is required to move from center to center. This adjustment should, however, be made empirically for each new set of capillary pipes.

Duration

Duration determines the length of time that a capillary pipe remains in a given position. When driven with an analog signal, the time spent in a position is determined simply by how long the voltage corresponding to that position is applied. For example, to move from pipe 0 to pipe 1 for 1 second, and then return to pipe 0, requires a voltage step of from 0 to 1 V that lasts 1 second, and then returns to 0 V.

If a digital signal is the external control, the stepper will stay in a position until it receives the next command. In the absence of a signal, the motor will go to the “0” position. Manually, the position is maintained until changed to another position.

Initializing the reference position

Before beginning any experiment it is required to initialize the reference position by pressing the INITIALIZE button. The stepper motor will move to whichever position is manually selected. This brings the pipes into a known position from which all other positions are referenced.

Initializing under external control

If the initialization is performed when there is 0 volts on the input line then the pipes will automatically assume the 0 position. If there is another voltage on the line then the pipes will go to that position immediately, and automatically, after the initialization. If at any time during an experiment or series of experiments the positions become confused then simply pushing the initialize button will bring everything back into register.

Fast stepping with Theta glass

Very fast perfusion stepping is possible using 2 barrel Theta tubing. The technique requires close attention to detail and careful placing of the pipes and the excised patch. The following is information provided as a general guide.

Preparation of the Theta glass

The tubing is pulled on a standard puller for a tip diameter of approximately 300 µm and a barrel to barrel spacing of approximately 100 µm.

Motor voltage adjustment

When using 100 µm steps, it is important to minimize any vibration produced by the stepper motor. This is accomplished by reducing the motor voltage with the control located on the rear panel.

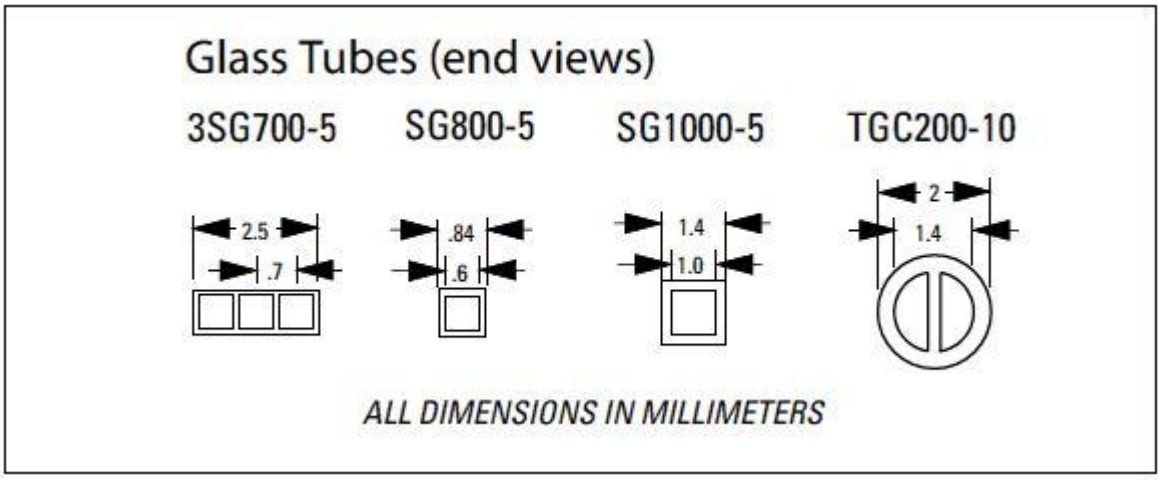
The voltage is lowered until the vibration artifact is minimized. Any residual artifact may be removed by subtracting averaged null traces.

APPENDIX

Specifications

Number of Steps	0 to 7 (2 to 8 positions)
Step Size	Adjustable from 100 - 1500 µm in 100 µm increments, with STEPS/POSITION switch
Step Speed	Typically 20 ms for a 700 µm step (~2.9 ms per 100 µm step)
Step Control	
Manual	8 position with POSITION selector
Analog Signal	8 positions with voltage levels 0-7 Volts, 1 V/step
Digital Signal	8 positions with 3 bit TTL signal
Maximum Stepped Range	12.5 mm
Mounting Handle	6.3 mm dia. x 10 cm long
Stepper Weight (including handle)	110.5 grams
Solution Manifolds Each system is supplied with three (3) manifold: The **SF-77C** and **SF-77CST** are supplied with two 6-to-1 and one 2-to-1 MM Series manifolds. The **SF-77CLT** is supplied with two 6-to-1 and one 2-to-1 ML Series manifolds.	
MM Series	MM-2, MM-4 and MM-6 manifolds use PE-50 tubing at the input and PE-10 tubing at output
ML Series	ML-2, ML-4 and ML-6 manifolds use PE-50 tubing at both input and output

Solution Flow Rates Rates are measured with a solution reservoir height of approximately 60 cm (24 inches).	
With MM Series	100 µl/min
With ML Series	1 ml/min
Control Box Size	4.37 cm x 21.26 cm x 15.24 cm (H x W x D)
Power Requirements	100-240 VAC, 50/60 Hz, 10 VA
Warranty	1 year, parts and labor



Reference

Jie Zheng and Fred Sigworth, Selecting Changes during Activation of Mutant Shaker Potassium Channels, *J. General Physiology*, 10:101-117, Rockefeller Univ. Press.

Cleaning/Maintenance

At the end of each experiment, allow all solutions to flow out of the reservoir and add a few ml of 70% ethanol. The manifold is composed of Delrin and stainless steel and PE-tubing is

unaffected by ethanol, thus this cleaning procedure can be withstood. Next flush all the lines with water. After all solutions have run through, remove the needle and dispose of the cotton tip (prevent cross contamination of solutions). Finally air dry (pressurized) solution lines.

Warranty and service

Warranty

The **SF-77C** is covered by our Warranty to be free from defects in materials and workmanship for a period of one (1) year from the date of shipment. If a failure occurs within this period, we will either repair or replace the faulty component(s). This warranty does not cover failure or damage caused by physical abuse. In the event that repairs are necessary, shipping charges to the factory are the customer's responsibility. Return charges will be paid by Warner Instruments.

Service

We recommend that all questions regarding service be referred to our Technical Support Department. We can be reached by phone at (800) 547-6766. email us at techsupport@harvardapparatus.com or through the web at http://www.warneronline.com.