

Applied Biophysics

P-FLOW PERISTALTIC PUMP

p-Flow Manual

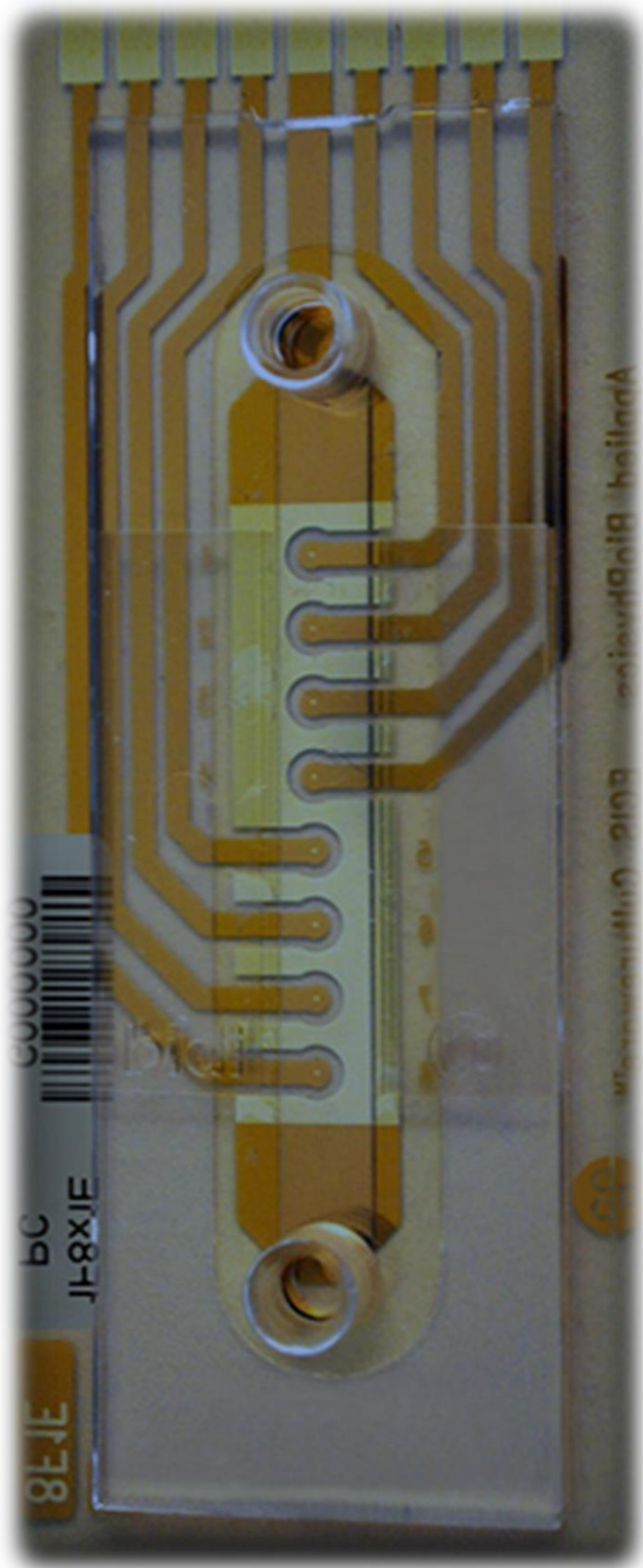


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P-FLOW PUMP & TUBING SPECIFICATIONS

Pump Specifications

- Maximum flow rate with 3.18mm ID tubing: 24ml/min
- Minimum flow rate with 3.18mm ID tubing: 70ul/min (1.2ul/sec)
- Flow rate resolution with 3.18mm ID tubing: 70ul/min from -20ml/min to +20ml/min
- Maximum ramp rate: 0-20ml/min in 150msec (forward or reverse)
- Full forward to full reverse: -24ml/min to +24ml/min: 400msec
- Pump speed: -60 to +60 RPM stepper driven motor
- Pump resolution: 1024 increments/rev
- Control input: USB
- Manual controls: forward/reverse, run/stop, speed control (not linear)
- Power: +15V DC, 1.2A, under 10W

Tubing Specifications

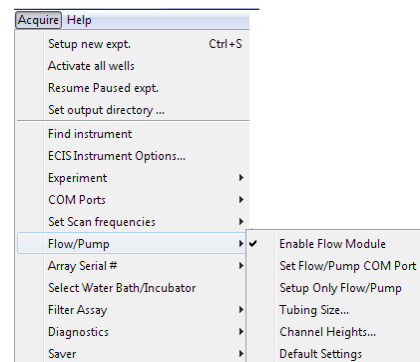
- Recommended (maximum) tubing ID: 3.18mm ID (1/8 in)
- Recommended (maximum) tubing OD: 4.7mm OD (3/16 in)
- 2-Stop Tubing Type: PVC (Polyvinyl Chloride) phthalate free tygon
- 2-Stop Tubing Size: 3.18mm ID, 4.7mm OD, wall thickness 0.76mm (1/32 in)
- 2 Stop Tubing Length: 455mm long with the stops separated by 105 mm.
- Catalog Number for ordering 2 stop tubing is: ECIS-pFT12
 - They are supplied in packages of 12 tubes.
- Bulk Tubing Type: Bioprene
- Bulk Tubing Size: 3.0mm ID, 4.0mm OD, wall thickness 1.0mm
- Bulk Tubing Length: 10m long – user can cut to desired lengths
- Catalog Number for ordering Bulk tubing is: ECIS-pFT10

1. PUMP SET-UP

NOTE: The pump generates between 5-10 watts of heat. Confirm the pump will not overheat your incubator by monitoring the incubator temperature.

If installing the pump in the incubator, please preheat the pump to eliminate condensation. Place the pump in a sealed plastic bag in the incubator to preheat.

- Plug the USB cable into the pump port and then plug the other end into a USB port on your ECIS Laptop or Computer.
- Go to **Acquire** → **Flow/pump** → **Enable Flow Module**
- Go to **Acquire** → **Flow/pump** → **Set Flow/Pump COM Port**
 - a. Choose the port that is indicated (Port 9)
- Go to **Acquire** → **Flow/pump** → **Tubing Size...**
 - a. Enter the tubing size(s) you plan to use.
These will now be available from a dropdown in the Flow Set-up. (3.18mm ID tubing is recommended and provided by ABP)
 - b. A pop-up box will appear asking for the flow rate at 60 RPM for 3.18 ID tubing
 - i. Enter this value: 24.0536
 - ii. Click **OK**
- Go to **Acquire** → **Flow/pump** → **Channel Height...**
 - a. Enter the channel height(s) of the arrays you plan to use not including the units (μm). These will now be available from a dropdown in the Flow Set-up Pump Menu.
 - b. We recommend you enter 360 and 660



NOTE: 1F8x1E and 1F8x10E are 360 μm . All other flow arrays are 660 μm .

- Go to **Acquire** → **Flow/pump** → **Setup Only Flow/Pump**
 - a. Pump menu will now appear on the right side of the ECIS main menu.
- Your pump is now connected and can be controlled through the ECIS Software

2. CHOOSING A FLOW PROGRAM

The p-Flow pump can be run in four modes: Computer Control – Single Speed, Computer Control – Multi Speed, Pulsing Flow, and Manual Control.

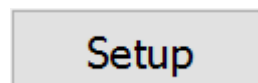
- **Computer Control – Single Speed:** Set a single flow rate for the entire experiment.
- **Computer Control – Multi Speed:** Set multiple flow rates to start and stop during an experiment.
- **Pulsing Flow:** This mode allows you to create pulsing flow to mimic blood vessels in vivo.
- **Manual Control:** Control the pump without a computer or ECIS system. Speed is controlled via a knob and a button starts and stops flow.

3. COMPUTER CONTROL – SINGLE SPEED

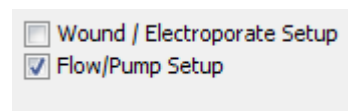
A single speed run will allow you to program a single pump speed to run continuously for the duration of the experiment. You can also start and stop the pump using the software controls as needed.

To create a program for a single continuous speed:

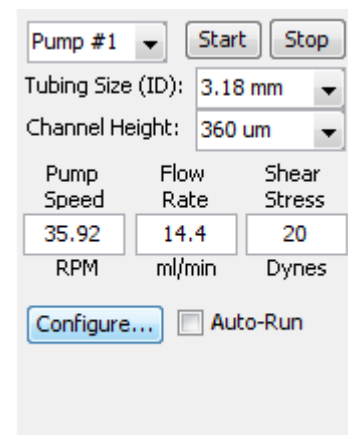
- Click the **Setup** button in the upper right side of the software.



- Find the **Flow Module** Check box.
 - Check the box to enable the module and begin setting up a run.



- Choose your **tubing size** and **channel height** from drop downs menus.



- Enter the desired **shear stress** in dynes cm² and the software will automatically calculate the correct **pump speed** and **flow rate** needed. You can also enter the desired **flow rate** and the software will calculate the **pump speed** and **shear stress**.

- In the pump menu, click **Start** to run pump and begin flow.

- Pump will begin running

- Set **Auto Run** to have the pump start when an ECIS measurement starts.

- To activate, check the box next to **Auto Run**.

- Click **Stop** to end your run and stop the pump.

4. COMPUTER CONTROL – MULTI SPEED

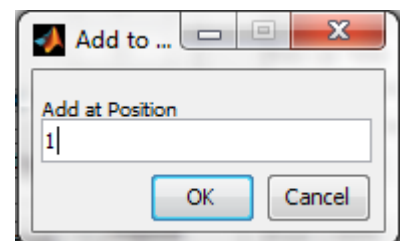
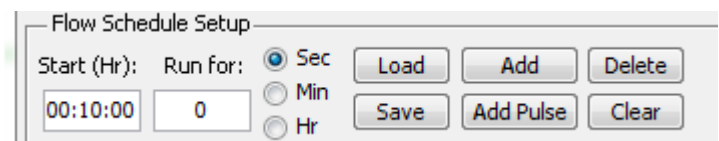
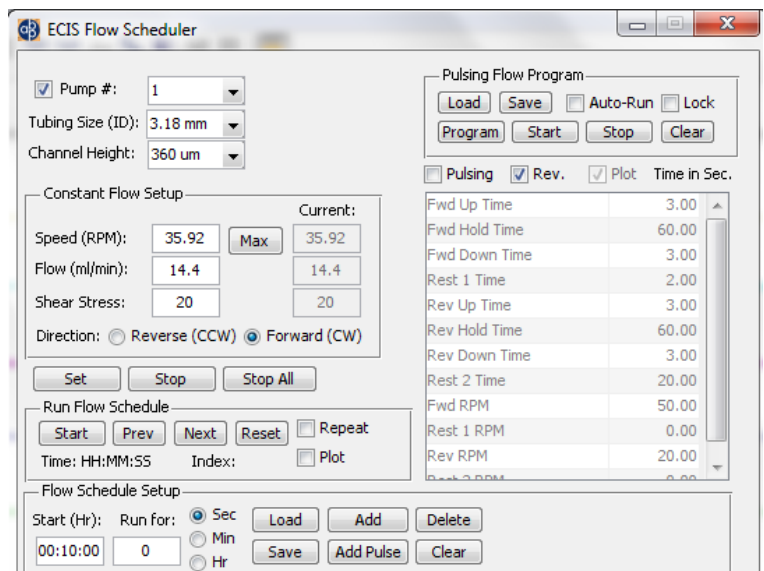
Once you have set-up a single speed run – you can move on to multiple speeds during a single experiment. This mode allows to run up to 25 different speeds during an experiment by creating a custom program.

Click **Configure** at the bottom of the pump menu. This will open an expanded pump control window called **ECIS Pump Scheduler**.



To create a program for multiple pump speeds:

- Choose the correct **tubing size** and **channel height** from the drop-down menus.
- Enter the desired **shear stress** in dynes cm^2 and the software will automatically calculate the correct **pump speed** and **flow rate** needed. You can also enter the desired **flow rate** and the software will calculate the **pump speed** and **shear stress**.
- Choose the direction of flow by clicking the button next to **Reverse** or **Forward**.
- Go to **Flow Schedule Setup**
 - Enter the total **Run Time** for these parameters.
 - Click the button next to **Sec, Min, Hr** to choose the units for the **Run Time**.
 - Click **Add** to add those parameters at position **1** in the sequence.
 - This will bring up a Box asking at what position to add the flow parameters. Click **OK** to confirm the position number.



- To add additional flow rates, repeat the steps above.

- Click **Add** to add those parameters at position **2** in the sequence.
- Repeat this process for position 3, 4, 5 etc. until you have all the required flow rates.

	Pump	Start At	Run For	RPM	MPM	Dynes	Pulsing
1	1	00:00:00	00:00:30	60.00	24.05	33.41	<input type="checkbox"/>
2	1	00:00:30	00:00:20	39.51	15.84	22.00	<input type="checkbox"/>
3	1	00:00:50	00:00:30	19.76	7.92	11.00	<input type="checkbox"/>

- You can add up to 25 different parameters into the program.

- Click **Save** to save the program sequence to be used at a later time.
- Click **Load** to load a previously saved program.
- Go to **Run Flow Schedule**.

- Choose **Repeat** to repeat the programmed sequence. *(Optional Step)*

Run Flow Schedule

Start Prev Next Reset Repeat

Time: HH:MM:SS Index: Plot

- This will repeat the entire programmed sequence continuously until **Repeat** is unselected.
- If **Repeat** is not selected – the program sequence will run through 1 cycle and then stop.

- Choose **Plot** to review flow profile. *(Optional Step)*
- Choose **Delete** to remove a programmed position. *(Optional Step)*
 - A window will pop up asking which position to delete
 - Enter position number and click **OK**
- Choose **Clear** to remove all programmed positions. *(Optional Step)*
 - All programmed positions will be removed
- Click **Start** to begin the program
 - The program will start with position **1** in the programmed sequence.
 - The sequence clock will begin counting.
 -

- If you need to skip to the next flow rate/position in the sequence before the programmed time choose **Next** and it will move to the next programmed flow rate.

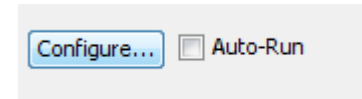


- Keep clicking the button until it gets to the desired position in the sequence.
- If you need to go back to a previous flow rate in the sequence choose **Prev** and it will move to the previous programmed flow rate.
 - Keep clicking the button until it gets to the desired position in the sequence.
- Click **Stop** to stop only the current programmed position.
 - If the pump is currently in position 1, it will stop flow until position 2 is set to begin. Then position 2 will begin and the cycle will continue until it is finished or it will repeat if that option is selected.
 - If there is only 1 position, the pump will stop flow permanently unless repeat is selected.
- Click **Reset** to stop the current program.
 - This will stop the pump and all sequence positions until **Start** is clicked again

5. PULSING FLOW

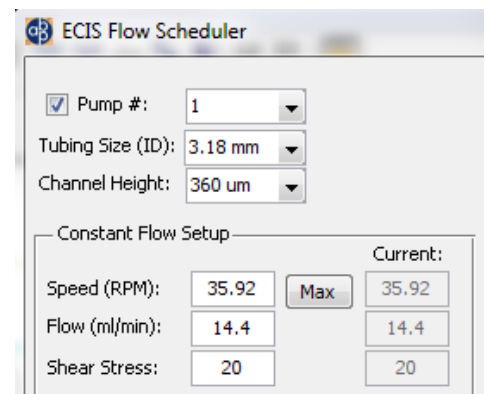
Once you have set-up a multi speed run – you can move on to pulsing flow during a single experiment. This mode allows to create pulsing flow which mimics the vessels in vivo. There are 2 pulsing modes – Forward and Forward + Reverse.

Click **Configure** at the bottom of pump menu. This will open an expanded pump control window called **ECIS Pump Scheduler**.



To create a program for pulsing flow:

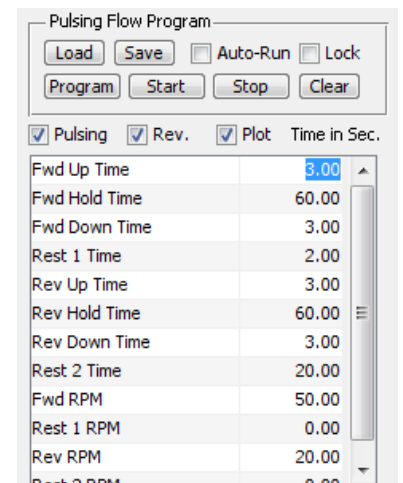
- Choose the correct **tubing size** and **channel height** from the drop downs menus
- Go to **Pulsing Flow Program**
 - Click **Pulsing** to program a pulse sequence.
 - Click **Plot** to view time line of the pulse sequence
- Enter the desired **shear stress** in dynes cm² and the software will automatically calculate the correct **pump speed** and **flow rate** needed. You can also enter the desired **flow rate** and the software will calculate the **pump speed (RPM)** and **shear stress**.
 - The pulse table only uses **RPM** – so you can use this to calculate the correct **RPM** to get the desired **Flow Rate** or **Shear Stress**.



NOTE: For Forward Only Pulsing there is a high speed (Fwd RPM) and a low speed (Rest 1 RPM)

To Create a Program for Forward Pulsing Flow:

- Go to the table and enter the desired times into the following rows
 - **Fwd Up Time** – Time to ramp up to desired **FWD RPM**
 - **FWD Hold Time** – Time the pump will hold constant **FWD RPM**
 - **FWD Down Time** – Time to ramp down to **Rest 1 RPM**



- **Rest 1 Time** – Time pump will run at **Rest 1 RPM**
- **Fwd RPM** – High **RPM** needed to get desired Flow Rate or Shear Stress
- **Rest 1 RPM** – Low **RPM** needed during rest period

NOTE: For Forward + Reverse Pulsing there is a forward speed (Fwd RPM) and a reverse speed (Rev RPM). The resting speed between pulses must always be ZERO (Rest 1 RPM, Rest 2 RPM).

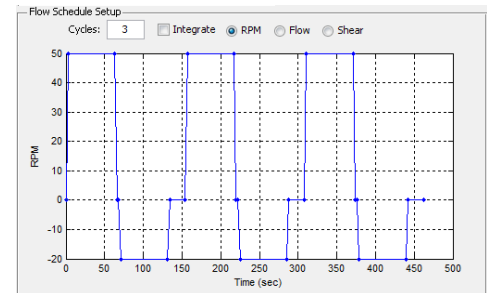
To Create a Program for Forward + Reverse Pulsing Flow:

- Go to the **Pulsing Flow Program** menu and click **Rev**
- Go to the table and enter the desired times into the following rows:
 - **Fwd Up Time** – Time to ramp up to desired **FWD RPM**
 - **Fwd Hold Time** – Time the pump will hold constant **FWD RPM**
 - **Fwd Down Time** – Time to ramp down to **Rest 1 RPM**

Parameter	Value
Fwd Up Time	3.00
Fwd Hold Time	60.00
Fwd Down Time	3.00
Rest 1 Time	2.00
Rev Up Time	3.00
Rev Hold Time	60.00
Rev Down Time	3.00
Rest 2 Time	20.00
Fwd RPM	50.00
Rest 1 RPM	0.00
Rev RPM	20.00
Rest 2 RPM	0.00

- **Rest 1 Time** – Time pump will run at **Rest 1 RPM**
- **Rev Up Time** – Time to ramp up to desired **Rev RPM**
- **Rev Hold Time** – Time the pump will hold constant **Rev RPM**
- **Rev Down Time** – Time to ramp down to **Rest 2 RPM**
- **Rest 2 Time** – Time pump will run at **Rest 2 RPM**
- **Fwd RPM** – **RPM** needed to get desired FORWARD Flow Rate or Shear Stress
- **Rest 1 RPM** – Must be set to 0.00
- **Rev RPM** – **RPM** needed to get desired REVERSE Flow Rate or Shear Stress
- **Rest 1 RPM** – Must be set to 0.00

- Choose **Plot** to review flow profile. *(Optional Step)*
- Click **Save** to save the pulsing program to be used at a later time.
- Click **Load** to load a previously saved program.
- Click **Program** to load profile into the pump and start pump running
 - Pump will begin running with pulsing program
- Click **Stop** to stop the pump.
 - This will stop the pump until **Start** is clicked



6. MANUAL CONTROL

Manual control allows you to use the pump without the ECIS System controlling the flow program and without a computer. You may set the pump speed manually using the knob on the back panel of the pump.

- Plug in power supply.
- Set toggle switch to run **Forward** or **Reverse**.
- Push **Run/Stop** button.
- Set desired speed using rotary pot on back of pump.
- **Pump indicator lights:**
 - **Green:** on-power, ready
 - **Red:** constant RPM in manual or under PC control
 - **Flashing Green:** program is loaded from PC, ready to run
 - **Flashing Red:** program is running
- To clear program, hold **Run/Stop** button on back of pump for 15 seconds.

NOTE: No connection to a PC or ECIS system is required to run in this mode.

Approximate pump RPM using rotary pot on back of pump:

Knob Position	RPM
A	0.1
B	1.5
C	3.5
D	7
E	10.5
F	12.5
G	14
H	17
I	25
J	35
K	45
L	55
M	60

7. FLOW KIT AND ARRAY SET-UP

The standard set-up for the p-Flow pump includes a 25mL Flow Kit comprised of the following parts:

Quantity Included in Kit	Flow Kit Item	Quantity Needed for set-up of 1 Array
2	25mL Glass Bottles	2
2	3 Port Bottle Cap	2
10	Luer Filter	1
1	Port Plug	1
4	Dip Tubes	4
12	2- Stop Tubing	1
10	Luer to Thread Connectors	4
10	Male-Male Tubing Adaptors	2
10	Red Lock Ring Adaptors	6
10	Luer to Luer Male adaptors	2
10m	Bioprene Tubing	4*

*pieces cut to length needed (~12 in)

To set-up an array for a flow assay follow the set-up guide below:

- Find 2 each of:
 - **Clear 25mL Glass Bottles**
 - **Blue 3-Port Bottle Caps**
- Assemble the bottles by screwing on the **Blue 3-Port Bottle Caps**
- Find 1 each of:
 - **Port Plug**
 - **Port Filter**
- Add the **Port Plug** to the non-threaded port on Bottle 1 (this is now the **2-Port Bottle**), add the **Port Filter** to the non-threaded port on Bottle 2 (this is now the **3-Port Bottle**)
 - The non-threaded ports appear slightly smaller than the threaded ports
 - Each cap has 1 non-threaded and 2 threaded ports
- Find the 4 **Dip tubes**.

- Add 1 **dip tube** to each of the open threaded ports on both bottle caps.
- Make sure they fit down all the way inside the cap.
- Find the **Luer to Thread Connectors**.
 - Add 2 **Luer to Thread Connectors** into the open ports on each of the blue bottle caps.
 - These will be the ports that also contain the **dip tubes**.
 - Make sure the **Luer to Thread Connectors** are tight inside the ports by pushing down.
 - All ports on both caps should be filled now.
- Find the **Red Lock Ring Adaptors**.
 - Add 4 **Red Lock Ring Adaptors** onto the luer locks of the **Luer to Thread Connectors** on both blue bottle caps.
 - Make sure the connections are tight by rotating the red portion.
- Find the **Bioprene Tubing** (10m box) and 2 **Red Lock Ring Adaptors**.
 - Cut 4 pieces approximately 12 in long of the **Bioprene Tubing**
 - Insert the prong end of 2 **Red Lock Ring Adaptors** into one end of 2 of the 4 pieces of cut **Bioprene Tubing**.
 - You should now have:
 - 2 pieces of tubing that have one end connected to a **Red Lock Ring Adaptor** and the other end empty
 - 2 pieces of tubing with nothing connected.
- Find the **2-Stop Tubing** and **Male-Male Tubing Adaptors**
 - You will need 1 length of **2-Stop Tubing** and 2 **Male-Male Tubing Adaptors**
 - Insert a **Male-Male Tubing Adaptor** into each end of the **2-Stop Tubing**.
- Connect the **2-Stop Tubing** to the cut **Bioprene Tubing**.
 - You will need the 2 pieces of **Bioprene Tubing** with nothing connected

- Insert one end of 1 cut length of **Bioprene Tubing** into one end of the **Male-Male Tubing Adaptors** connected to the **2-Stop Tubing**.
- Repeat with the other cut length of **Bioprene Tubing**
- You should now have both of the cut lengths of **Bioprene Tubing** attached to each end of the **2-Stop Tubing**.
- Make sure the tubes are tightly connected to each adaptor.

- Connect the **2-Stop Tubing** to the pump head
 - Insert the middle portion of the **2-Stop Tubing** into the pump head.
 - ***Make sure the stops are not inside the pump head***
 - Push down on the silver lever to lock it in place
- Place the **2-Port Bottle** with the plug on the right of the pump.
- Place the **3-Port Bottle** with the filter on the left of the pump.
- Find the two pieces of tubing each with one **Red Lock Ring Adaptor** attached.
 - Insert the empty end of one piece of the cut tubing onto a **Red Lock Ring Adaptor** on the **3-Port Bottle**.
 - Insert the empty end of one piece of the cut tubing onto a **Red Lock Ring Adaptor** on the **2-Port Bottle**.
- Find the two ends of tubing connected to the pump head
 - Insert the empty end of the left piece of the cut tubing onto the red lock ring on the **3-Port Bottle**.
 - Insert the empty end of the right piece of the cut tubing onto the red lock ring on the **2-Port Bottle**.
- Insert your prepared array into the array holder.
 - Clamp Array in place.
- Find the **Male Luer to Luer Adaptors**.
 - You will need 2 **Male Luer to Luer Adaptors**.

- Insert **Male Luer to Luer Adaptors** into each of the two reservoirs.
- Connect the bottles and tubing to the array
 - Insert the **Red Lock Ring Adaptor** from the tubing on the **3-Port Bottle** to the luer lock closest to the array contact pads.
 - Insert the **Red Lock Ring Adaptor** from the tubing on the **2-Port Bottle** to the luer lock farthest to away from the array contact pads.
- Turn on the instrument and software.
 - Set the pump to the proper settings (*Refer the Flow Program Sections*)
 - Turn on the pump and begin flow.
- Check to make sure the media in both bottles is staying at the same level.
 - Media in the **2-Port Bottle** will rise slightly and then stop.
 - If the media continues to rise or fall steadily, turn off the pump and make sure everything is properly aligned and tightened. The rise and fall of media indicates an air leak at one of the connections.
- Check for leaks around each lock ring
 - If there is a visible leak stop the pump and make sure everything is properly aligned and tightened
 - Push down on the luer locks if necessary.
- If no leaks occur you are now ready to begin a Time Course Experiment with ECIS

8. INNOCULATION OF ARRAYS

The p-Flow module allows one to study cells under flow condition while simultaneously making ECIS measurements. The heart of this system is the ECIS Flow array that has ECIS electrodes on the floor of a flow channel.

The suggested protocol listed below is that suggested by **ibidi Integrated BioDiagnostics**, the manufacturer of the channel used with the ECIS flow arrays.

- Prepare your cell suspension at the desired concentration. Depending upon your cell type, application of 100,000 cells per cm² growth area should result in a confluent layer within the channel in 2-3 days. When seeding cells, fill only the correct channel volume into the channel. Avoid surplus cell suspension in the reservoirs.
- Refer to the table below for recommended seeding protocols for each type of flow array.
- If desired, the floor of the channel can be pre-coated with protein following the information provided in the appendix of this manual.

Array Type	Growth Area per Channel	Channels per Array	Recommended Cells per Channel	Recommended Seeding Density	Max. Well Volume Channel/Reservoirs (µL)	Channel Height (µm)
1F8x1E PC	2.5 cm ²	1	110,000 – 250,000	1.2 – 2.5 x10 ⁶	90/60	360
1F8x10E PC	2.5 cm ²	1	110,000 – 250,000	1.2 – 2.5 x10 ⁶	90/60	360
6F1E PC	0.6 cm ²	6	30,000 – 60,000	0.7 – 1.4 x10 ⁶	50/60	660
6F10E PC	0.6 cm ²	6	30,000 – 60,000	0.7 – 1.3 x10 ⁶	50/60	660
1F2Y8x10E PC	2.8 cm ²	2**	140,000 – 280,000	0.8 – 1.6 x10 ⁶	180/60	660

** Array can be used on 2 different sides. (30 & 45 degree bifurcated sides)

- Method 1: Apply cell suspension (***suspension volume should be = to the maximum channel volume for array type***) into the end of the channel allowing capillary action to fill the narrow space. Quick dispensing helps to avoid trapped air bubbles. Inject 60µl of cell-free medium simultaneously into each reservoir at the ends of the channel to avoid flushing out the cells; avoid tilting the flow slide to prevent unwanted removal of cells from the channel.
- Method 2: Apply cell suspension (***suspension volume should be = to the maximum channel volume for array type***) and then place the flow slide in the incubator for the time required for cells to attach to the surface. Once the cells are in place, add 60µl of medium into each reservoir.

Use the following medium exchange protocol:

- Empty the reservoirs completely without emptying the channel.
- Inject the new media solution into one reservoir and remove the old solution from the other side. Make sure the old solution is completely replaced. For a ~99% exchange add about 3 x the channel volume from one side.

NOTE: In order to avoid air bubbles inside the channels fill the channels by putting the pipet tip directly to the channel's inlet. Apply the volume with a constant and swift flow. When using very small volumes, it may be necessary to fill the channel with a low volume syringe (1.0 - 2.5 ml).

APPENDIX

Array Type	Electrodes per Channel	Channels per Array	Electrode Area (mm ²)	Maximum Number of Cells Measured	Max Well Volume Channel/Reservoirs (μL)	Channel Height (μm)
1F8x1E PC	8 x 1	1	0.049	50-100	90/60	360
1F8x10E PC	8 x 10	1	0.49	500-1000	90/60	360
6F1E PC	1	6	0.049	50-100	50/60	660
6F10E PC	10	6	0.49	500-1000	50/60	660
1F2Y8x10E PC	8 x 4*	2**	0.49	500-1000	180/60	660

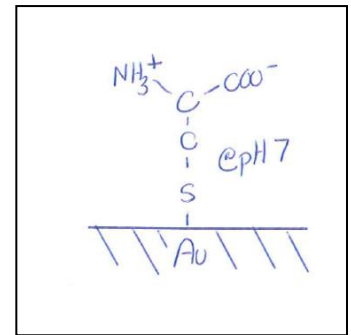
*Four electrodes are equal in size to 8W10E

** Array can be used on 2 different sides. (30 & 45 degree bifurcated sides)

CYSTEINE TREATMENT

Our preferred way to clean the electrodes is with the amino acid **L-cysteine**. This treatment cleans and modifies the electrode surfaces enhancing experimental repeatability with minimum variation between ECIS wells.

To accomplish this, the well should be flooded with a 10 mM sterile solution of L-cysteine in distilled H₂O. The solution can easily be prepared in your laboratory and filter sterilized, or the solution can be purchased from Applied BioPhysics*. After exposure to the cysteine, the solution can be rinsed out of the wells, proteins adsorbed if desired, and the wells inoculated with cells. The time for the cysteine exposure depends upon the history of the array, for relatively newly etched arrays often the reaction is completed in only a few minutes, but to be certain the reaction has gone to completion, an hour-long exposure is recommended for all arrays.



The cysteine will form a covalent sulfur-gold linkage with the electrode surface likely displacing any unwanted small molecules that have adsorbed to gold surface over time and stabilizing the impedance. This cysteine layer provides a hydrophilic substrate that is excellent for protein adsorption and ultimately cell attachment and spreading.

Flow arrays: Add 100ul for 15 minutes

NOTE: You can purchase *Electrode Stabilizing Solution in 50mL or 250mL bottles.*

- **50mL – Catalog Number: C-001**
- **250mL – Catalog Number: C-002**

COATING WITH PROTEINS

ECIS electrodes shipped from Applied Biophysics have no macromolecular coatings. When culture medium is added to the arrays, proteins and other large molecules in the medium immediately adsorb to the very wettable gold surface as they do with any uncoated tissue culture dish. Often one wishes to define the substrate by coating it with proteins – for example collagen, gelatin, fibronectin or other ECM proteins.

We suggest using the following protocol: Prepare a solution of the desired protein at 100 micrograms per ml or more in 0.15M NaCl. We have found that the use of phosphate buffer (PBS) can seriously interfere with the adsorption of some proteins, so if a buffer is required, a mild Tris solution (e.g. 0.01M) is recommended. The electrode arrays are stable under acidic conditions, so when coating with collagen there is no problem using solutions containing acetic acid.

To coat the electrode, place the protein solution in the bottom of the well for 10 minutes or more. If the protein is valuable, lower protein concentrations can be used with increased times for adsorption.

Once adsorption has taken place, an approximate mono-molecular layer of the protein will coat the surface. You can safely rinse the protein solution from the well with sterile medium, saline or water without concern of removing the adsorbed layer. Medium can now be added and inoculation begun.

Remember, the first macromolecules the substrate is exposed to will be the final coating that the cells first encounter.

NOTE: We do not recommend drying the protein solutions in place as it can foul or damage the electrodes.