Sutter Instrument Company – P1000 Micropipette puller – Pipette cookbook 2018

**PARAMETER SETTINGS**

The P-97 and P-1000 puller use the following parameters to control the melting and pulling of the glass: Each application described in the Pipette Cookbook will offer a starting program where the heat, pull, velocity, time/delay and pressure settings are suggested. Please see the SutterInstrument YouTube Channel to find the Scientists Empowering Scientists Webinar, “How to Make Better Pipettes”, which describes the parameters settings in more depth. The manual that came with your puller will also have a description of the parameter settings. A brief description of each setting is discussed below:

**HEAT** – This is the amount of current supplied to the filament. The value does not represent the temperature, but indicates how much current is delivered to the filament. The filament needs to get hot enough to melt the glass, and this heat value is dependent on the filament shape, the filament size and the size and wall thickness of the glass. If you use a heat setting that is too high or too low, you risk burning out the filament or damaging the puller. So it is not a good idea to guess at the heat setting. You can also introduce a lot of variability if the heat setting is not ideal. To determine the proper and safe HEAT value to use, one should run a RAMP TEST. For more information about the Ramp TEST, see page 12.

**PULL** – This is the hard pull introduced to the glass after the glass has softened. This pull value determines the amount of current supplied to the pull solenoid to create the hard pull. The puller can pull the glass out with a hard pull (Pull = < 0 >) where the puller only uses the gravitational weight of the plunger inside the puller to pull on the glass. A pull of < 0 > is typical when pulling a patch pipette where you want a 3-5mm short taper and a 1-3µm tip. A pull of 50-150 is typical when making a sharp electrode of 30 to100 MΩ or when making a microinjection needle.

**VELOCITY** – This is the rate of separation of the puller bars when the glass first starts to melt. The velocity is detected by a transducer inside the puller, a patented feature, and the velocity has a direct correlation to the viscosity of the glass. The velocity is the “trip point” for turning off the heat and starting the cooling and the hard pull. When the velocity is low, the pipette taper will be shorter. When the velocity value is low enough, as when pulling a patch pipette, the puller will pull in multiple cycles or “LOOPS”. A velocity of 18 - 65 is typical when pulling a patch pipette, and when using a one-line program, the lower the velocity is, the more times the program will loop. LOOPING is further discussed on page 29. A pull of 50-150 is typical when making a sharp electrode of 30 to100 MΩ or when making a microinjection needle.

**TIME** – This is the duration of air used to cool the glass and the filament as the glass is being pulled. When using the Time mode, the glass softens, the velocity trip point is reached, and then the glass is cooled at pulled simultaneously. The duration of cooling is determined by the value of the Time where each unit of time is equivalent to 0.5 milliseconds of cooling air. Traditionally a Time of 150 (75ms) is recommended when using a trough filament or when using thin walled glass and making a pipette for slice patch recording. A higher Time value of 250 (125ms) is recommended when using a box filament and pulling thick walled glass.

**DELAY** – This is the alternative mode of cooling which provides a longer duration of cooling (300ms) than the Time mode (max of 127.5ms). In the Delay mode, the glass softens, the velocity trip point is reached, and then 300ms of cooling is initiated. The Delay value determines how long the glass is cooled before the hard pull is engaged and continues cooling the glass as the glass is pulled. So by cooling the glass while delaying the hard pull, you can determine how viscous the glass is when it is being pulled. I find this to be a more sophisticated way of controlling the glass when making electrodes. A low Delay value of 40-90 units will expose the glass to less cooling before the hard pull, the glass will be more molten when the hard pull engages and this will result 11 in a longer tapered pipette. A higher delay value of 100-250 will provide more cooling to the glass before the hard pull engages, the glass will be more viscous, and this will result in a shorter tapered pipette. The delay mode of cooling is also most often used when pulling patch pipettes to deliver a longer duration of cooling to the glass which helps produce shorter tapered patch pipettes. As demonstrated on pages 30 and 31, a Delay mode of with a Pull value of results in short tapered pipettes.

**PRESSURE** – This is the pressure of air used to cool the filament and the glass. The default Pressure setting is 500 units, which represents 2psi of cooling air. Both the Time and Delay modes (duration of cooling) work in conjunction with the Pressure to cool the glass. The higher the Pressure, the more robust the cooling is to the glass and filament. An increase in Pressure will cool the glass faster and shorten the taper. A decrease in the Pressure will reduce the cooling to the glass and allow for longer and more gradual tapers.

**General Guideline for the Parameter Settings**

When designing a program or adjusting your existing parameter settings, it is quite easy to end up “lost” and with very unstable settings if you do not know where to start or when a setting is considered too high or too low. If you are trying to design a program from scratch, please refer to the “cookbook” programs provided for various applications. There you will find what we hope proves to be a good starting point. If you are in the midst of adjusting and fine-tuning your existing parameter settings, below is a general guideline suggesting the range of settings to stay within for each parameter. These ranges are a general rule of thumb, and there could often be exceptions depending on the filament and glass combination, the OD and ID of glass you are using, and the final morphology of the pipette you are aiming for.

**HEAT:**

Ramp - 5 to Ramp + 15 for most applications!

**PULL:**

0 Patch, 3-5mm taper and 1-3µm tips

30 -70 Microinjection, 6-8mm tapers, 0.9 to 0.5µm tips

70 - 150 High MΩ, 9-15mm tapers, 0.5 to 0.06µm tips

**VELOCITY:**

20 - 60 Patch, 3-5mm taper and 1-3µm tips

50 - 80 Microinjection, 6-8mm tapers, 0.9 to 0.5µm tips

70 - 100 High MΩ, 9-15mm tapers, 0.5 to 0.06µm tips

**TIME:**

250 box, 150 trough Patch, 3-5mm taper and 1-3µm tips

250 box, 150 trough Microinjection, 6-8mm tapers, 0.9 to 0.5µm tips

250 box, 150 trough High MΩ, 9-15mm tapers, 0.5 to 0.06µm tips

**DELAY:**

1 Patch, 3-5mm taper and 1-3µm tips

60 - 110 Microinjection, 6-8mm tapers, 0.9 to 0.5µm tips

40 - 90 High MΩ, 9-15mm tapers, 0.5 to 0.06µm tips

**PRESSURE:**

200 - 500 Thin Walled Glass

200 - 700 Thick Walled Glass

**RAMP TEST**

To choose an appropriate heat setting, you must first determine the amount of heat required to melt your glass by running a RAMP TEST. The heat value established by the ramp test will depend on the type of heating filament installed in your puller and the type and dimension of glass you are using. The ramp test value for a box filament will traditionally be 1.5 to 2 times higher than the value of a trough filament.

**When to Run a Ramp Test**

* Using the Puller for the First Time
* Whenever you Change the Filament
* Whenever you Change Glass
* Before Writing or Editing a Program

**How to Run a Ramp Test on a P-97 (for the P-1000, press “Ramp” on upper right of display)**

* Enter any program number <0-99> when using a P-97
* Press clear <CLR> to enter the control functions
* Press <0> to not clear all parameter values
* Press <1> to run a RAMP TEST
* Install glass and press <PULL>

*To interrupt the RAMP TEST or reset the display after a ramp test, press <RESET>*

**When a ramp test is executed, the following events take place**

1. The puller increments the HEAT

2. Once the HEAT output allows the glass to soften, the puller bars will drift apart

3. When the factory-set ramp velocity is reached (trip-point) the heat is turned off

4. The Ramp Test value will be shown on the display

**Expected ramp test values**

Filament # Filament Dimensions Expected Ramp Test Values Maximum Heat

FT330B 3mm x 3mm TROUGH 250 – 300 (see warning below) Ramp + 20

FB255B 2.5mm x 2.5mm BOX ~ 480 to 540 Ramp + 30

FB330B 3.0mm x 3.0mm BOX ~ 550 to 650 Ramp + 40

FB245B 2.5mm x 4.5mm BOX ~ 750 to 880 Ramp + 75

***\*Warning*** *- If the ramp test value for your trough filament is OVER 300 units, this might be too high and could indicate that the filament shape is poor and therefore inefficiently heating the glass.*

*Please remove your filament and reshape it according to the instructions in Chapter 14.*

**Recommended Heat Settings for Each Filament Type**

Filament Recommended Heat Setting

3mm TROUGH Ramp +15

2.5mm x 2.5mm BOX Ramp

3.0mm x 3.0mm BOX Ramp

2.5mm x 4.5mm BOX Ramp

***\*Caution*** *- If your Heat setting is greater than 20 to 50 units above the ramp value, depending*

*on the filament shape and size, (see above table), you will risk burning out the filament!*