

Standard Operating Procedure (S.O.P) for Two-Electrode Voltage Clamp in *Xenopus laevis* Oocytes

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This S.O.P will direct you in the procedure of the well-established electrophysiological technique known as two-electrode voltage clamp (TEVC). TEVC is extremely useful for measuring the total membrane current produced by ion channels or non-ion-channel receptors in a single cell. Non-ion-channel receptors that TEVC could evaluate are metabotropic receptors that are coupled to ion channels through intracellular pathways and neurotransmitter transporters that generate intrinsic currents during ligand binding.

Among the studies that can be performed are [1] pharmacological analyses to determine the drug's pharmacological properties, such as agonism/antagonism, potency, and efficacy; [2] to identify the receptor's basic biophysical and electrogenic properties, and [3] to evaluate the effect of mutagenesis in receptor dynamics. Furthermore, TEVC can be applied to receptors in their native environment and heterologous expression systems, such as muscle tissue and oocytes harvested from the African clawed frog *Xenopus laevis*, respectively. This S.O.P. uses Molecular Devices' instruments to recollect data on *X. laevis* oocytes.

In TEVC, the oocyte is impaled by voltage-sensing and current-injecting electrodes. The membrane potential, measured by the voltage-sensing electrode and a high input impedance amplifier, is compared with a command voltage (holding potential), and the difference is brought to zero by a high-gain feedback amplifier. In addition, the injected current is monitored via a current-to-voltage converter, which measures the population response.

Different factors could affect the current response obtained from TEVC experiments and, consequently, the parameter to be determined. The parameters include temperature, solution flow rate, degradation of the silver chloride wires, degradation of the pipette electrode, and biological differences among *Xenopus* oocytes batches, among others.

Important:

- ***ALWAYS touch the faraday cage or the stainless steel tabletop BEFORE touching the Headstages to discharge any charge accumulation in your hands.***
- ***Follow the specific order to turn on/off the equipment.***
- ***Do not touch the gold piece without gloves when assembling/disassembling the electrode holders from the headstage. Dirty pins must be cleaned with a kimwipe damped in 70% alcohol.***
- ***Electrode resistance should be between 0.5 – 5 M Ω , with the resistance of the voltage electrode being higher than the current electrode.***
- ***Be sure the Ag/AgCl electrodes are optimal. If the Ag/AgCl wire (electrode holder) is in bad shape, scratch the wire with sandpaper and electrodeposit AgCl using 100 mM HCl and a power supply/battery (9 V). If the Ag/AgCl pellet (ground electrode) is color black, sand the pellet until the color is flat gray.***
- ***Using fire-polished borosilicate capillary tubes is preferable because they produce less damage to the Ag/AgCl wire in the electrode holder.***
- ***Vertical and horizontal capillary tube pullers could be employed satisfactorily.***
- ***The glass electrode tip size and taper shape are key factors in impaling oocytes. For xenopus oocytes, the glass electrode must be balanced between gradual and uniform taper to cause less damage during impalement and short taper to increase the stiffness required to penetrate***

the rigid membrane.

- ***Air bubbles must be removed from the KCl solution in the glass electrode. This is because air bubbles increase noise during recordings and affect the proper connection between the Ag/AgCl electrode and the glass electrode tip.***
- ***If cRNA-injected oocytes will be employed, it is recommendable to voltage clamp a few oocytes in MOR-2 buffer before the first experiments to ensure the oocytes are in good condition. Checking oocytes in MOR-2 avoid wasting drugs and time.***
- ***Just take a few xenopus oocytes (2–3 oocytes) from the incubator at once. Leaving the oocyte out of the incubation for extended periods affects the quality of the oocyte.***

Materials:

- Waste Container with Bleach (2% sodium hypochlorite)
- Kimwipes
- 2–1000 µL Micropipette and Tips
- 50 mL Serological Pipet
- 50 mL Disposable Conical Tubes
- Disposable Plastic Bulbs (8 mL, wide tip)
- 5 mL Luer Lock Syringe with a Female Luer-to-Female Luer Connector
- Silver (Ag) Wires (PFA-uncoated; 0.254 mm diameter)
- Ag/AgCl Pellet (2 mm diameter, 4 mm long)
- Capillary Tubes with Filaments (Borosilicate, 1.2 mm O.D./ 0.94 mm I.D.)
- MOR-2 Buffer (*see Appendix A*)
- 100 mM HCl (stored at room temperature; *see Appendix A*)
- 3 M KCl in Syringe with a Microfil Flexible Needle Filled (needle: 28G; stored at room temperature; *see Appendix A*)
- Xenopus Oocytes Nanoinjected with cRNA of the Protein of Interest (incubated at 17 °C)
- Power Supply (5–12 V) or a 9 V Battery
- Glass Electrode Storage Vessel (WPI E215) or a 100 mm Petri Dish with Non-hardening Modeling Clay (preferably Crayola)
- Perfusion Control System (Warner Instruments: VCS-8-PINCH)
- Oocyte Perfusion Chamber (Warner RC-1Z)
- Capillary Glass Tube Puller (Horizontal or Vertical)
- Two Glass Electrode Holders (Molecular Devices: HL-U)
- Two Micromanipulators Mounted in Magnetic Base for Amplifier Headstages
- Dissection Microscope (2–5X magnification roulette)
- Analog-to-Digital Converter (Molecular Devices, Digidata model)
- Amplifier (Molecular Devices, Axoclamp 2B or 900A or Geneclamp 500B)
- Computer with Clampex Software
- *Optional:* Oscilloscope
- *Optional:* External Filter (Frequency Devices 900)

Procedure:

Pre-Experiment Procedure:

- a) Day Before:
 1. Prepare MOR-2. See *Appendix A* for the recipe.
 2. Prepare drug stock solutions if they are required for the experiment.

3. Prepare 3 M KCl, if it is necessary. See *Appendix A* for the recipe.
 4. Prepare 100 mM HCl, if it is necessary. See *Appendix A* for the recipe.
 5. Check the Ag/AgCl wires in the electrode holders are in optimal condition. If they are not, follow the instructions below. See *Appendix B* for an illustration of the HL-U holder.
 - Unscrew the pin cap of the electrode holder to remove the Ag/AgCl wire, and scratch the wire with sandpaper to remove the AgCl layer. **Place the pin cap, threaded collar, gold pin, and the rest of the holder in a safe place.**
 - Check the power supply (5–12 V) is off and connect the scratched Ag wire to the negative pole. A 9 V battery can be used instead of the power supply. Connect the positive pole to another Ag wire. Turn the power supply on and dip both electrodes in a 100 mM HCl solution as much as possible to cover a larger area of the wire with AgCl. The AgCl layer is flat gray. **Do not touch the electrode with each other.**
 - After 5 seconds, remove the electrode from the HCl solution, turn off the power supply, and clean the electrodes with reverse osmosis H₂O.
 - Carefully insert the Ag/AgCl wire through the silicon seal and bend the wire 90° at the silicon seal to make better contact with the gold pin. Then, reassemble the electrode holder back.
 6. Check that the electrode holder's cone washer is appropriate for the glass electrode diameter (1.2 mm). The washers are color-labeled (clear, I.D. 1.3 mm).
 7. Check the Ag/AgCl pellet in the oocyte chamber (ground electrode) is in optimal condition. If not, clean the pellet with sandpaper until the color is flat gray.
 8. Check the perfusion and electrophysiological protocols to be used in Clampex.
- b) Day of Experiment:
1. Prepare the waste container with 2% bleach for decontamination. **Oocytes to be discarded must be decontaminated in this container for at least 30 minutes.**
 2. Turn on the electrophysiological rig, as the *Turning On the Electrophysiological Rig* section explains.
 3. Assemble the perfusion line with MOR-2 first, then check a few noninjected and injected oocytes before preparing any drug solution. This strategy ensures the oocytes are in good condition for voltage clamping. Follow the instructions in the *Assembling Perfusion Lines*, *Assembling the Electrodes*, and *Voltage Clamping Procedure* sections.
 4. Prepare 50 mL of drug solutions, if required.
 5. Prepare the folder where the data will be saved.

Turning On the Electrophysiological Rig:

- a) Axoclamp 2B:
(Voltage Headstage: HS-2A x1LU, Current Headstage: HS-2A x10MGU, Oscilloscope: required, External Filter: required)
1. Turn on the Perfusion Control System and check the *Operation Mode* is MANUAL.
 2. Be sure the A/D Converter power switch is in the “On” position.
 3. Turn on the External Filter
 4. Turn on the Oscilloscope
 5. Turn on the Computer and open Clampex software.
 6. Turn on the Amplifier, turn all knobs to their minimum values, and check the following setting,
 - *I Display Select* → 0.1 x I₂
 - *H1* → x1
 - *H2* → x10

- *Outputs*: 10Vm, 1m OUTPUT BANDWIDTH → 30
 - *Mode* → BRIDGE
 - *Microelectrode 1 (ME1)*: INPUT OFFSET → 5.0
 - *Microelectrode 2 (ME2)*: INPUT OFFSET → 5.0
7. Let the equipment warm up for at least 30 minutes. In the meantime, prepare the drug solutions and fill in the perfusion lines.
- b) Geneclamp 500B:
(Voltage Headstage: HS-2A x1LU, Current Headstage: HS-2A x10MGU, Oscilloscope: optional)
1. Turn on the Perfusion Control System and check the *Operation Mode* is MANUAL.
 2. Be sure the A/D Converter power switch is in the “On” position.
 3. *Optional*: Turn on the Oscilloscope
 4. Turn on the Computer and open Clampex software.
 5. Turn on the Amplifier, turn all knobs to their minimum values, and check the following setting,
 - *Mode* → SETUP
 - *Scaled Output* → I2
 - Voltage Clamp: TEST buttons → lights off
 6. Let the equipment warm up for at least 30 minutes. In the meantime, prepare the solutions and fill in the perfusion lines.

Assembling Perfusion Lines:

This protocol is designed to reduce air bubbles in the perfusion line, which affect the perfusion rate among lines and the parameters to be determined. See *Appendix B* for an illustration of the perfusion system Warner VCS-8PINCH.

It is recommendable to voltage clamp oocytes in MOR-2 buffer before assembling the drug perfusion lines. This strategy ensures the oocytes are in good condition and avoids wasting expensive drugs.

- a) Determine how many perfusion syringes (60 mL luer lock syringe) will be employed in the experiment.
- b) *Optional*: Prepare any solution other than MOR-2 buffer that will be employed during the experiment.
- c) Assemble the manifold and switch over the unused perfusion lines in the manifold with the supplemental plastic rod that comes with the manifold. ***Unused lines must be removed because backfilling occurs during perfusion, affecting the perfusion rate.***
- d) Attach the c-flex tube of each perfusion line to its corresponding pinch valve. ***Be sure the c-flex is properly inserted to the back of the valve.***
- e) Check the stopcocks of the perfusion syringes are open.
- f) Set the *Operation Mode* of the perfusion system control unit to MANUAL and close any open pinch valve.
- g) Disassemble the 18G blunt needle (pink luer) from the stopcock of the perfusion syringes to be used.
- h) Transfer MOR-2 buffer to a 50 mL disposable conical tube.
- i) Fill with MOR-2 buffer the 5 mL luer lock syringe assembled with the female luer-to-female luer connector. ***Be sure no air bubbles are in the syringe and the connector (except the tip).***
- j) Attach the female luer connector to the “Main/Wash” perfusion stopcock and transfer the MOR-2 buffer to the perfusion syringe. Remove any air bubbles by pulling and pushing the 5 mL syringe plunger a few times. ***Leave a little bit of solution in the 5 mL syringe, and be sure no***

air bubbles are in the stopcock.

- k) Detach the female luer connector and attach the pink blunt needle to the stopcock. Fill the perfusion syringe with MOR-2 buffer to the 10 mL mark.
- l) *Optional*: If other perfusion syringes need to be filled, repeat steps *j)* through *k)*.
- m) Fill the “Main/Wash” perfusion syringe with MOR-2 Buffer to the 60 mL mark.
- n) Open the pinch valve of the “Main/Wash” perfusion line and carefully fill the oocyte chamber (Warner RC-1Z), ***avoiding spilling MOR-2 buffer.***
- o) Check the stopcock between the vacuum and the oocyte chamber outlet is closed and start the vacuum.
- p) Simultaneously, open the “Main/Wash” valve and the chamber outlet stopcock. ***Check no spill of MOR-2 buffer has occurred.***
- q) Remove any air bubbles in the perfusion line by flicking the bubble with your fingernail.
- r) Close the “Main/Wash” valve first, followed by the chamber outlet stopcock.
- s) *Optional*: If other perfusion lines need to be filled, repeat steps *p)* through *r)*. ***Let the MOR-2 buffer flow until the solution reaches the upper top of the syringe luer.***
- t) Remove any air bubbles in the oocyte chamber using a 200 μ L micropipette + tip.
- u) Check for leaks or spills in the lines, manifold, and oocyte chamber. If leaks are found, verify the c-flex tube and line connections are not loose. Clean any spill.
- v) Voltage clamp a few oocytes to check if the oocytes are in good condition. Then, follow the instructions in the *Assembling the Electrodes* and *Voltage Clamping Procedure* sections.
- w) *Optional*: Fill each perfusion syringe with its respective solution to the 50- or 60-mL mark. Open the pinch valve and the chamber outlet stopcock simultaneously, and let the solution flow for ~5 mL before closing the valve and the outlet stopcock. Lastly, wash any remaining solution from the oocyte chamber by perfusing ~5 mL of the “Main/Wash” perfusion line.
- x) Check for leaks or spills in the lines, manifold, and oocyte chamber. If leaks are found, verify the c-flex tube and line connections are not loose. Clean any spill found.
- y) Set the *Operation Mode* of the perfusion system control unit to ANALOG and close the stopcocks of any perfusion line with an open pinch valve.

Assembling the Electrodes:

- a) If the electrode holders are not assembled with the amplifier’s headstages, insert them. ***Be sure the gold pin in the electrode holder is the right size.***
- b) See *Appendix C* to select the appropriate parameters (or protocol) for producing glass electrodes using a capillary glass tube puller.
- c) Place the capillary glass tube symmetrically around the heating element of the puller and begin the pulling process. Once the glass breaks, carefully place each glass electrode in the glass electrode storage vessel. Alternatively, you can place the glass electrodes in a petri dish containing a clay bump in the middle. ***Pull no more than three capillary tubes consecutively to avoid variance in the glass electrode shape and tip dimension.***
- d) Take a glass electrode and fill it with 3 M KCl solution with a syringe with the microfil flexible needle. After removing the needle, gently tap the glass electrode with the fingernail to eliminate any air bubbles. Add enough KCl solution to contact the Ag/AgCl wire in the electrode holder. ***The flexible needle is fragile, be careful not to break it.***
- e) Check the pipette cap of the electrode holder is loose and carefully insert the glass electrode, avoiding scratching the AgCl layer as much as possible. Tighten the pipette cap to stabilize the glass electrode.
- f) Repeat this procedure for the second electrode.
- g) After both glass electrodes are assembled, submerge the electrode tip into the oocyte chamber

solution to avoid the solidification (crystals) of the KCl solution.

Voltage Clamping Procedure:

An oscilloscope or the Membrane Test tool in Clampex (Tools → Membrane Test) are required to set the voltage clamp gain and stability (phase lag) in Geneclamp 500B and Axoclamp 2B, respectively. If the Membrane Test tool is employed, some parameters must first be defined in the Membrane Test Setup (Configure → Membrane Test Setup) and in the Membrane Test window. **The Membrane Test tool could also be used for Geneclamp 500B, but it is not explained in this S.O.P.**

a) Axoclamp 2B: (oscilloscope required)

1. Move the glass electrodes as far as possible from the oocyte chamber RC-1Z using the course movement knobs of the micromanipulators.
2. Using a wide-tip disposable plastic bulb, place an oocyte in the largest hole of the oocyte chamber (left hole) and open the vacuum briefly to fix the oocyte into the hole.
3. Check all settings are at their minimum values
4. Immerse both glass electrodes into the oocyte chamber solution and position them close enough to the oocyte to impale it as soon as the amplification settings are ready. Use the course movement knobs of the micromanipulators.
5. Press the BRIDGE button in the *Mode* section to set the amplifier in bridge balance mode.
6. Adjust the INPUT OFFSET knob in the *Microelectrode 1 (ME1)* section to bring the Vm (mV) digital meter to 0 mV.
7. Adjust the INPUT OFFSET knob in the *Microelectrode 2 (ME2)* section to bring the V2 (mV) digital meter to 0 mV.
8. Set the Channel 1 of the oscilloscope: voltage at 50 mV/div and time at 10 ms/div. Set the Channel 2: voltage at 20 mV/div and time at 10 ms/div. Set the trigger mode to external.
9. Activate the Membrane Test tool in Clampex and press Alt 1 to run Test 0, which sends a –10 mV square pulse to the voltage electrode (ME1). Determine the ME1 resistance by adjusting the BRIDGE knob in the *Microelectrode 1 (ME1)* section until the square signal change to a straight line. **The resistance is calculated by multiplying the knob value by 10 and must have a value of 0.5 – 5 MΩ.**
10. Press Alt 2 to run Test 1, which sends a –10 mV square pulse to the current electrode (ME2). Determine the ME2 resistance by adjusting the BRIDGE knob in the *Microelectrode 2 (ME2)* section until the square signal change to a straight line. **The resistance is the knob value and must be 0.5 – 5 MΩ.**

Important: The resistance of the voltage electrode (ME1) must be larger than the current electrode (ME2). If that is not the case, remove both electrodes from the oocyte chamber and switch the whole electrode holders from one headstage to the other.

11. Press the STOP button in the Membrane Test window to turn off the current electrode (ME2) test pulse.
12. Before impaling the oocyte, check the GAIN, PHASE LAG (ms), MULTIPLIER, and HOLDING POSITION knobs in the *Voltage Clamp* section are at their minimum values (fully counterclockwise).
13. Impale the oocyte with the voltage electrode (ME1) using the fine movement knob of the micromanipulator, observing the Vm meter until a big jump in the voltage (in tens of mV) is detected.
14. Impale the oocyte with the current electrode (ME2) in the same way the ME1 was impaled but by observing the V2 meter.
15. Turn the HOLDING POSITION knob in the *Voltage Clamp* section until the two RMP

BALANCE led are balanced and equally dim to set the holding potential to resting potential (zero net current). This is the best holding potential for adjusting the voltage clamp GAIN and PHASE LAG.

Important: The oocyte membrane potential (V_m meter) should be at least -30mV or more hyperpolarized (more negative potential values).

16. Press the TEVC button in the *Mode* section to set the amplifier in voltage-clamp mode.
17. Press Alt 3 to run Test 2 in the Membrane Test tool of Clampex to send a -10 mV square pulse to the current electrode (ME2).
18. Set the MULTIPLIER knob of the *Voltage Clamp* section to 0.1 ms.
19. Turn the GAIN and PHASE LAG (ms) knobs iteratively to set the voltage clamp gain and avoid oscillation in the current electrode during fast and significant current responses. Use the Membrane Test window of Clampex to follow the progress.
 - Slowly increase the GAIN knob and note that the output signal begins to look like a capacitive transient. Stop when oscillations are observed.
 - Adjust the PHASE LAG knob until the oscillations disappear.
 - Continue increasing the GAIN and PHASE LAG knobs until the maximum value of the GAIN has been reached (fully clockwise), or the PHASE LAG control reduces the speed of the clamp too much to be useful.
20. Press the STOP button in the Membrane Test window to turn off the current electrode (ME2) test pulse.
21. Adjust the HOLDING POSITION knob in the *Voltage Clamp* until the V_m digital meter display -70 mV .
22. Start the electrophysiology experiment by pressing RECORD in Clampex.
23. At the end of each experiment, return all settings using the inverse procedure.
 - Return the HOLDING POSITION to the resting membrane potential (RMP BALANCE balanced).
 - Reduce the GAIN knob to its minimum value.
 - Reduce the PHASE LAG knob to its minimum value.
 - Set the MULTIPLIER knob to Off.
 - Press the BRIDGE button to escape from voltage clamp mode.
 - Return the HOLDING POSITION to its minimum value.
 - Move the current electrode (ME2) as far as possible from the oocyte chamber.
 - Move the voltage electrode (ME1) as far as possible from the oocyte chamber.
24. Transfer the oocyte from the oocyte chamber to a waste container.

If you expect to take a break between experiments, submerge the electrode tip into the oocyte chamber solution to avoid the solidification (crystals) of the KCl solution.

- b) Geneclamp 500B: (oscilloscope required)
1. Move the glass electrodes as far as possible from the oocyte chamber RC-1Z using the course movement knobs of the micromanipulators.
 2. Using a wide-tip disposable plastic bulb, place the oocyte in the biggest hole of the oocyte chamber (left hole) and open the vacuum briefly to fix the oocyte into the hole.
 3. Check all settings are at their minimum values.
 4. Immerse both glass electrodes into the oocyte chamber solution and position them close enough to the oocyte to impale it as soon as the amplification settings are ready. Use the course movement knobs of the micromanipulators.
 5. Press the SETUP button in the *Mode* section to set the amplifier to current clamp mode.
 6. Press the V1 button in the *DC Meters* section to observe V1 on the upper digital meter.

7. Press the I2/V2 button of the *DC Meters* section to observe V2 on the lower digital meter.
8. Press the ZERO V1 button in the *Electrode #1* section to force the voltage electrode (#1) to zero potential. It should have a value of 0 mV on the upper DC meter.
9. Press the ZERO V2 button in the *Electrode #2* section to force the current electrode (#2) to zero potential. It should have a value of 0mV on the lower DC meter.
10. Press the R1 button in the *DC Meters* section to measure the voltage electrode resistance on the upper DC meter. **Must have a value of 0.5 – 5 MΩ.**
11. Press the R2 button in the *DC Meters* section to measure the current electrode resistance on the upper DC meter. **Must have a value between 0.5 – 5 MΩ.**
Important: The resistance of the voltage electrode (#1) must be larger than the current electrode (#2). If that is not the case, remove both electrodes from the oocyte chamber and switch the whole electrode holders from one headstage to the other.
12. Press the V1 button of the *DC Meters* section to observe V1 on the upper DC meter.
13. Press the I2/V2 button of the *DC Meters* section to observe V2 on the lower DC meter.
14. Before impaling the oocyte, check the following settings,
 - GAIN, STABILITY, and HOLDING POTENTIAL knobs in the *Voltage Clamp* section are at their minimum values (fully counterclockwise).
 - TEST buttons in the *Voltage Clamp* section are unselected (lights off).
 - SETUP button in the *Mode* section is selected (light on).
 - (-) button in the *Voltage Clamp* section is selected (light on).
 - I2 button in the *Scaled Output* section is on (light on).
15. Impale the oocyte with the voltage electrode (#1) using the fine movement knob of the micromanipulator, observing the V1 meter in the *DC Meters* section until a big jump in the voltage (in tens of mV) is detected.
16. Impale the oocyte with the current electrode (#2) in the same way the voltage electrode was impaled but by observing the V2 meter in the *DC Meters* section.
17. Turn the HOLDING POTENTIAL knob in the *Voltage Clamp* section until the two RMP BALANCED LEDs are balanced and equally dim to set the holding potential to resting potential (zero net current). This is the best holding potential for adjusting the voltage clamp GAIN and STABILITY.
Important: The oocyte membrane potential (V1 meter) should be at least -30mV or more hyperpolarized (more negative potential values).
18. Press the VOLTAGE CLAMP button in the *Mode* section to set the amplifier in voltage-clamp mode.
19. Press the FREQ arrowhead buttons in the *Scaled Output* section until the 1k label is lighted. This set the low-pass filter to 1 kHz.
20. Press the GAIN arrowhead buttons in the *Scaled Output* section until the x1 V/μA label is lighted.
21. Press the FAST TEST button in the *Voltage Clamp* section to apply square wave pulses of -10 mV to the oocyte membrane.
22. Turn the GAIN and STABILITY knobs iteratively to set the voltage clamp gain and avoid oscillation in the current electrode during fast and significant current responses. Use an oscilloscope or the Membrane Test window of Clampex to follow the progress.
 - If an oscilloscope is used, set the voltage to 50 mV/div, the time to 20 ms/div, and the trigger mode to inline.
 - Slowly increase the GAIN knob and note that the output signal begins to look like a capacitive transient. Stop when oscillations are observed.

- Adjust the STABILITY knob until the oscillations disappear.
 - Continue increasing the GAIN and STABILITY knobs until the maximum value of the GAIN has been reached (fully clockwise), or the STABILITY control reduces the speed of the clamp too much to be useful.
23. Press the FAST TEST button in the *Voltage Clamp* section to turn off the membrane test pulse.
24. Adjust the HOLDING POTENTIAL knob in the *Voltage Clamp* until the V1 digital meter (upper meter) in the *DC Meters* section displays -70 mV.
25. Start the electrophysiology experiment by pressing RECORD in Clampex.
26. At the end of the experiment, return all settings using the inverse procedure.
- Return the HOLDING POTENTIAL to the resting membrane potential (RMP BALANCED LEDs balanced).
 - Reduce the GAIN knob to its minimum value.
 - Reduce the STABILITY knob to its minimum value.
 - Press the SETUP button to escape from voltage clamp mode.
 - Return the HOLDING POTENTIAL to its minimum value.
 - Move the current electrode (#2) as far as possible from the oocyte chamber.
 - Move the voltage electrode (#1) as far as possible from the oocyte chamber.
27. Transfer the oocyte from the oocyte chamber to a waste container.

If you expect to take a break between experiments, submerge the electrode tip into the oocyte chamber solution to avoid the solidification (crystals) of the KCl solution.

Post-Experiment Procedure:

- a) Carefully invert any remaining oocyte into the waste container with bleach and let the bleach solution rest for at least 30 minutes. Then, dispose of the solution using the proper waste container and discard the materials in a biological waste (trash can with red bag).
- b) Set the *Operation Mode* of the perfusion system control unit to MANUAL, and check all pinch valves are closed.
- c) Close the vacuum and perfusion lines stopcocks. Next, remove the c-flex tubes from the pinch valves.
- d) Turn off the equipment in the following order,
 1. Turn all knobs and buttons of the Amplifiers to their initial settings and turn the Amplifier off.
 2. Perfusion Control System.
 3. *Optional*: External Filter.
 4. *Optional*: Oscilloscope.
- e) Open the vacuum and open the stopcocks of the filled perfusion lines to discard the solutions.
Avoid spilling.
- f) Remove the glass electrodes and discard them in a sharp-object container.
- g) Remove the electrode holders from headstages and rinse the Ag/AgCl wire with reverse osmosis water.
- h) Wash the perfusion lines and oocyte chamber by filling each syringe three times with reverse osmosis water. Check the Ag/AgCl pellet is also washed.
- i) When the perfusion lines are empty, disengage the manifold from the oocyte chamber and disassemble it in two parts. First, insert the biggest part of the manifold into a beaker, insert the plungers into the syringes and apply pressure to “air dry” the lines. Next, dry the oocyte chamber using the vacuum.

- j) The working area and any material should be cleaned at the end.
- k) Be sure the rest of the equipment is shut down: lights, capillary tube puller, and computer. Return any material and equipment to their proper place. The pipettes must be returned to their maximal volume.

Appendix A:

MOR-2 Solution (pH = ~7.4, Osm: mOsm)						
Reagent	Company	Cat. No.	State	MW (g/mol)	Concentration (mM)	Mass / 4 L (g)
NaCl	Sigma Aldrich	S5886	Solid	58.44	82.5	19.2852
KCl	Sigma Aldrich	P5405	Solid	74.55	2.5	0.7455
MgCl ₂ · 6H ₂ O	Sigma Aldrich	M2670	Solid	203.30	5	4.0660
Herpes · Na	Sigma Aldrich	H3784	Solid	260.29	3.5	3.6441
Na ₂ HPO ₄	Sigma Aldrich	S5136	Solid	141.96	1	0.5678
Hepes	Sigma Aldrich	H4034	Solid	238.30	1.5	1.4298
EGTA	Sigma Aldrich	E3889	Solid	380.35	0.5	0.7607
CaCl ₂ · 2H ₂ O	Sigma Aldrich	21097	Solid	147.01	0.2	0.1176

3 M KCl Solution for Electrodes						
Reagent	Company	Cat. No.	State	MW (g/mol)	Concentration (M)	Mass / 100 mL (g)
KCl	Sigma Aldrich	P5405	Solid	74.55	3.0	22.3650

~100 mM HCl Solution for AgCl Electrode position						
Reagent	Company	Cat. No.	State	MW (g/mol)	Concentration (mM)	Volume _{aq} / 50 mL (μL)
HCl	Sigma Aldrich	H1758	Liquid (~36.5–38%)	36.46	100	414.7

Appendix B:

Figure #1: Perfusion System – Warner Instruments VCS-8PINCH

Components of a Pinch Valve System

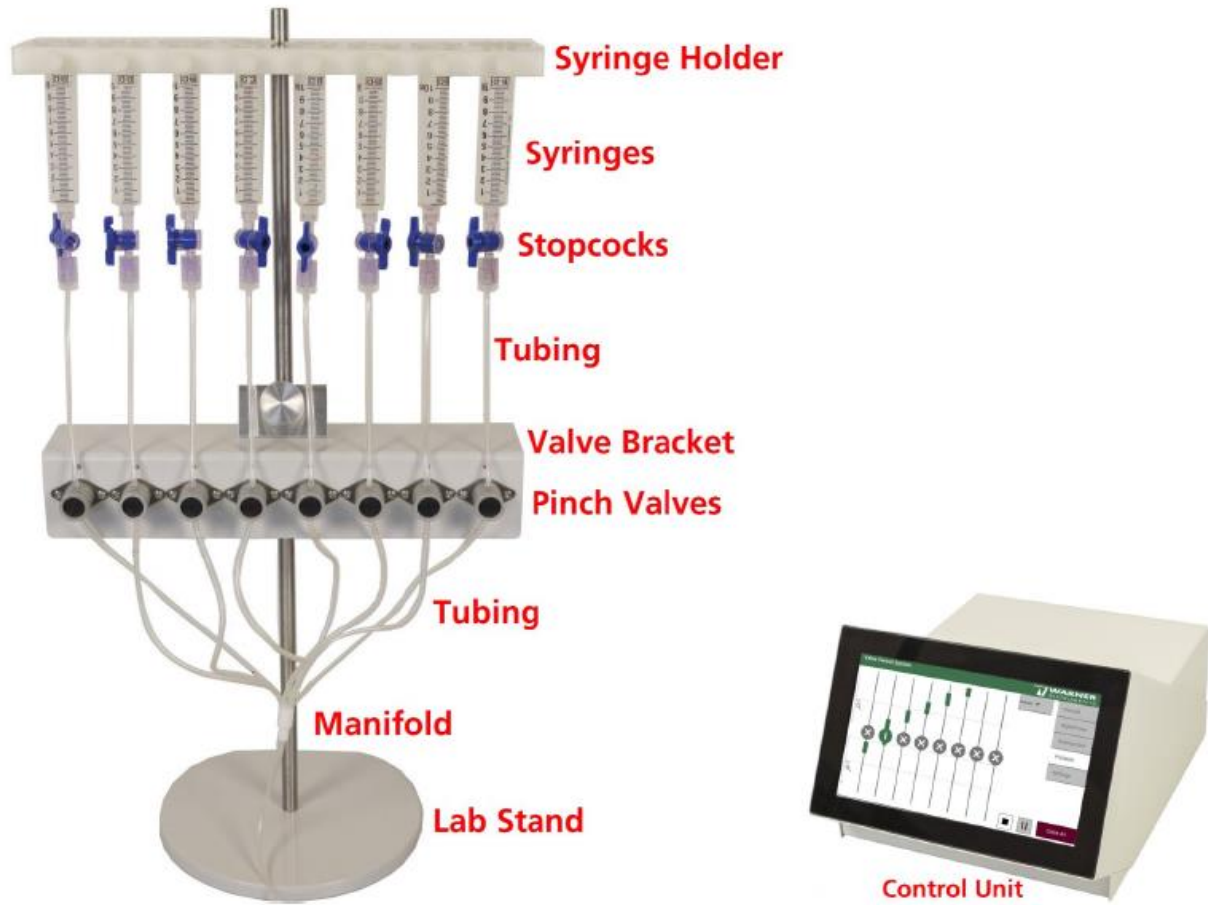
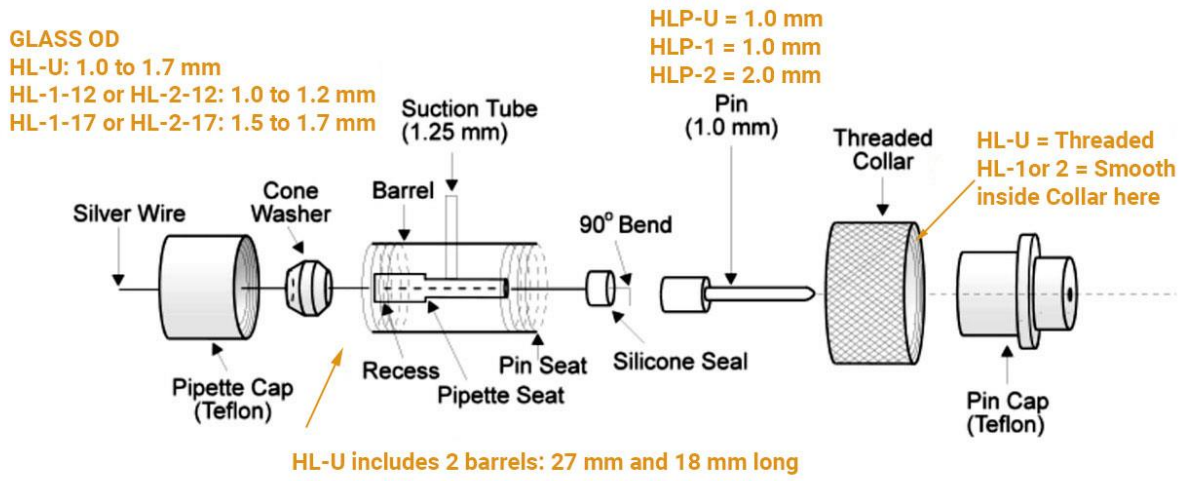


Figure #2: Electrode Holder – Molecular Devices HL-U



Appendix C:

Company	Model	Type	Protocol	Heat	Filament	Velocity	Delay	Pull
Sutter Instruments	P-2000	Horizontal	42	380	4	35	200	83
Company	Model	Type	Protocol	No. 1 Heater	No. 2 Heater			
Narishige	PC-10	Vertical	Step 1	-	68%			