ISAKSON LAB
Vasoreactivity Protocol

Make Filtered Krebs-Ca

1. Krebs stock is in large jug and 2L bottles on counter.
2. Add 100uL of 1.0M $CaCl_2$ solution to each 50mL Krebs to reach final concentration of 2mM Ca^{2+}.
3. Adjust pH to 7.4.
4. Fill 50mL syringe w/ Krebs-Ca and cap and filter with 0.22um PES filter. Each rig needs 30-40mL.
5. Top 2 x 5mL syringes with cut-off 200uL pipette tips and fill with filtered Krebs-Ca.

Set up Vasoreactivity Rig

1. Collect glass pipet tips, sutures, forceps for sutures, screwdriver, tubing, alligator clamp, and rubber gaskets.
2. Face left (non-adjustable) side towards you, "right" (silver adjustment knob) side back, with vertical rods to the left. Cut ledge of insert should point to the front.
3. Slide in glass micro-pipette top on front side and focus scope on it with highest magnification.
4. Break off micro-pipette tip at "correct" size of under 50um diameter – this will be quite close to the end.
5. Use a 200uL pipette-tipped syringe to perfuse a tube with filtered Krebs-Ca.
6. Attach Krebs-filled tube to back of micro-pipette and perfuse micro-pipette, clearing the tip. Avoid air bubbles. Leave syringe attached and set aside, elevated.
7. Rotate rig 180 degrees (rods on right), add the second micro-pipette, and break off the tip.
8. Clamp off the end of the first tube just before the syringe with an alligator clamp and remove syringe.
9. Use syringe to fill a second tube with filtered Krebs-Ca, then use filled tube to perfuse second micro-pipette. Avoid air bubbles.
10. Push first micro-pipette tip (left side of rig, away from you) almost halfway across the center chamber and tightening silver knob (not too tight). Pull black o-ring across bars on either side to hold micro-pipette in place.
11. Push second tip (right side of rig, facing you) towards center, but leave plenty of room in between micro-pipette tips. Tighten silver knob just to the point of resistance – it needs to be able to move still.
12. On right side of rig (facing you), move small black bar over micro-pipette. Use mini screwdriver to tighten silver screw gently to hold micro-pipette in place.
13. Use the silver knob on end of right side (facing you) to move the right micro-pipette tip forward just short of touching, then align micro-pipette tips by adjusting X/Y axes using the other knobs around the central well for the X-axis, tightening the well in place with the black knob. Use the black horizontal wheel under the left (facing away from you)

micro-pipette to adjust the Y-axis for that tip to match the right tip. (If alignment is beyond range of motion, unlock left micro-pipette, spin it a bit and re-attach to try again.)

14. Add filtered Krebs-Ca to the well, filling to a level above the micro-pipette tips.
15. Slip on sutures (3 onto the right side, facing you, and 2 onto the left side, away from you) and tie them down gently farther onto the pipet than the vessel will reach, where it's wider.
16. Pull pipette on the right back, using the silver knob, to make hanging a vessel easier.
17. If you leave and come back, cap or cover liquids to prevent particulate or dust contamination.

Rig Imaging Setup (for Rigs 1 & 3)

1. Turn on computer.
2. Put bottles in heating box, with blue tops on (P1 tight, P2 loose).
3. Plug in heater cord and turn on both boxes in back.
4. Turn on blue pressure transducer pump
5. Set P1 to 150 mmHg (F1 → Pressure menu) and P2 = 1. Status + ENTER to turn on. (Leave on this menu; ENTER will toggle on/off from here on). Flow speed should be 10.
6. Check tubes to verify no bubbles, then loosen P1 lid. Set P1 and P2 to 45 mmHg, turn status to "off" by hitting ENTER. Lights on top will turn off when status changes to "off." (To turn back on, tighten lid 1 before hitting "ENTER".)
7. Tube marked "drug" goes into bath beaker, "waste" tube goes into waste beaker, and "bath in" into waste beaker.
   *Rig 2 is the same except that the little container replaces big ones, tube plugs into Big Ben instead of pressure transducer box to control pressure, and connect to short metal tube (long metal tube goes into liquid).
8. Log into computers (Isakson/gapjunction).
9. Open Myoview software and select "pressure myography" from two options.
10. Click "capture" on "camera" tab. "Start data collection" will make this tab appear if it's missing.
11. The knob on the back of the scope controls light intensity. Turn it bright enough to light the whole field.
12. Draw two boxes in the field of view to set zones for measurement.
13. Hit the "new sheet" button at the top of the screen. Click and drag tab to slide to view both side-to-side.
14. Right-click the graph space and select "zone 1." Right click again and select "inner diameter." Repeat for zone 2.
15. Set time length to 5:00 so that it starts zoomed in.
16. Click the sine curve button and set vertical limite to the vessel size (e.g. 200um for MAs) to set grid sizing.
17. Hit "DMT204CM" software shortcut for temperature control and wait for it to find the box. It should already be set to 37 degrees C. Red = not plugged in, yellow = heating, green = at temp.

Cannulate Vessel

1. Handle vessels gently by the ends only (better yet, by bit of fat next to the end).
2. Drop vessel into Krebs-Ca in well of rig.
3. Handling from end of vessel with smallest forceps, pull onto micro-pipette tips, starting with the 3-suture side.
4. Slip sutures down over vessel and tie down snugly, don't pull too hard or sutures will break.
5. Check for leaks in vessel.

Perform vasoreactivity experiment

1. Carry rig over to vasoreactivity station, holding syringe elevated above rig.
2. Load rig onto scope, lock in place with black bar.
3. Find vessel on camera and focus.
4. Pinch top of tube, move to P1 beaker, pressurize to 45 mmHg before releasing. P2 stays clamped.
5. Attach bath outflow to back and bath inflow to front. Clear excess liquid and turn on bath inflow for 5 minutes to replace the liquid in the bath. Outflow should drain to "waste" beaker.
6. Hit "start data collection."
7. Set lines to match diameter (green = outer; red = inner). Use coarse/fine movements.
8. At the end of 5 minutes, move outflow from waste beaker into "bath" beaker to create a circular flow.
9. Increase pressure to 80 mmHg.
10. Hold syringe up.
11. Tighten P1 lid, hit "ENTER" to start.
12. Pinch off tube for P1 (currently in syringe) at the syringe end and plug immediately into P1 port on pressure transducer, where the little loop of tubing is currently connected.
13. Make sure the lid on P1 is tight and that the vessel hasn't collapsed.
14. Pinch P2 at the far end and unclamp (pinch outside of clamp, at edge) and plug far end into P2 port on transducer. Tighten P2 lid.
15. Plug in heat and plug in "bath out," etc., as described above.
16. Instructions for serial dilution and drug curves are posted by the vasoreactivity benches.
17. To mark timepoints of drug addition, etc., double-click to "enter comment." Add a comment at initial max dilation for animal information, then to measure "pre-NS309," "1uM NS309," at appropriate timepoints, etc.