INSTRUCTION MANUAL

Models 800A, 805A

In vitro Test Apparatus for 300C and 305C Muscle Lever Systems

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1.0 Introduction

The 800A and 805A *in vitro* test systems are used with 300C/300C-LR and 305C/305C-LR muscle lever systems respectively. These test systems enable physiology researchers to easily test intact muscle tissue with an ASI muscle lever system. The 800A and 805A consist of a vertical mounting plate, legs, motor mount, tissue bath, vertical translation stage for the bath, lower muscle clamp, two platinum stimulation electrodes, electrode holder and a vertical translation stage for the lower clamp. Also included are an oxygenating bubbler, tube clamp, motor cable clamp, screws to mount the motor to the motor mounting plate and a set of metric Allen keys.

All parts are manufactured from corrosion resistant materials (anodized aluminum, stainless steel and Delrin). The vertical mounting plate is an extrusion with vertical T-slots that allow additional equipment to be mounted on the plate. These T-slots also permit components to be repositioned on the mounting plate by simply loosening a screw and sliding the component to the new position. Extra T-nuts and screws can be purchased from ASI.

The 800A and 805A provide flexibility to the researcher by including a vertical translation stage for the tissue bath that allows the bath to be easily raised or lowered for access to the tissue. The position of the bath with respect to the lever arm and with respect to the lower tissue clamp can be adjusted with stops located on the vertical mounting plate.

The location of the lower tissue clamp can be adjusted through a wide range by using the dual coarse/fine vertical translation stage connected to the lower tissue clamp assembly. The fine position control allows the resting tension or resting length of the muscle tissue to be easily and accurately set.

The position of the electrode clamp can also be adjusted over a wide vertical range by simply loosening the clamp screw and repositioning the electrode clamp anywhere along the length of the lower tissue clamp support tube.

1.1 Specifications

Mounting Apparatus

Type:	IPS anodized extruded aluminum "T" profile.	
Accessories Included:	metric Allen key set, motor cable clamp, water circulation tube clamp.	
Dimensions:	320mm W x 250mm D x 520mm H	
Tissue Clamp Movement: (Vertical direction)	Coarse:	18mm/turn 42mm total travel
	Fine:	2.3mm/turn 2mm total travel
Bath Movement: (Vertical direction)	800A:	70mm nominal 200mm maximum
	805A:	100mm nominal 200mm maximum

Bath

Type:	Radnoti Tissue-Organ Bath with Removable T.N.V. Dispenser Tube
Model:	800A: 1583-51 805A: 1583-101
Accessories Included:	1 Luer valve, 2 Luer connectors, 1 Teflon needle valve
Dimensions:	800A: 51mm OD x 34mm ID x 75mm Depth (110mm overall length) 805A: 75mm OD x 51mm ID x 90mm Depth (95mm overall length)
Volume:	800A: 50ml 805A: 100ml
Max. Muscle Length:	800A: 45mm 805A: 55mm
Temperature Control:	Baths are fully water-jacketed to provide stable temperature control and incorporate quick disconnect fittings.

Stimulation Electrodes

Holder:	Delrin
Circuit Board:	Electrodes soldered to circuit board, terminal block to connect wires to BNC connector
Type:	Pure Platinum Bar
Dimensions:	800A: 2mm Wide x 30 AWG Thick x 50mm Long 805A: 2mm Wide x 30 AWG Thick x 65mm Long

2.0 Apparatus Setup

2.1 Unpacking

Unpack the apparatus from the shipping container. The 800A/805A comes completely assembled except for the glass tissue bath that is shipped in a separate box within the main shipping container. Care must be taken when handling the tissue bath. Enclosed in a second box are the set of metric Allen keys. Remove all parts from the shipping container.

Figure 1 Photo of 800A with 300C Attached.

Position the apparatus on a lab bench as shown in Figures 1 and 2.

Figure 2 Photo of 805A with 305B Attached.

2.2 Attaching the Motor

Before mounting the motor to the 800A/805A attach the lever arm to the motor with the arm oriented horizontally and pointing to the left when viewing the motor from the shaft end. The lever arm can also be attached or repositioned after the motor is attached to the motor mount plate.

Mount the motor (4) on the Motor Mount (5) with the shaft of the motor pointing towards the right side of the apparatus (pointing towards the lower tissue clamp) and the lever arm pointing out from the vertical plate, see Figure 3. The correct screws are provided for mounting the motor (the 300C mounts with M3 x 0.5 x 12mm socket head cap screws and the 305C mounts with M4 x 0.7 x 16mm socket head cap screws). Remove the four motor mount screws from the package and attach the motor to the plate using the metric Allen keys provided. Two sets of mounting holes are provided in the motor mount plate. The smaller set are for the 300C and the other set for the 305C.

Use the black plastic Cable Clamp (2) located on the left of the motor to clamp the motor cable to the Vertical Mounting Plate (1). The clamp can be repositioned to any convenient location if so desired.

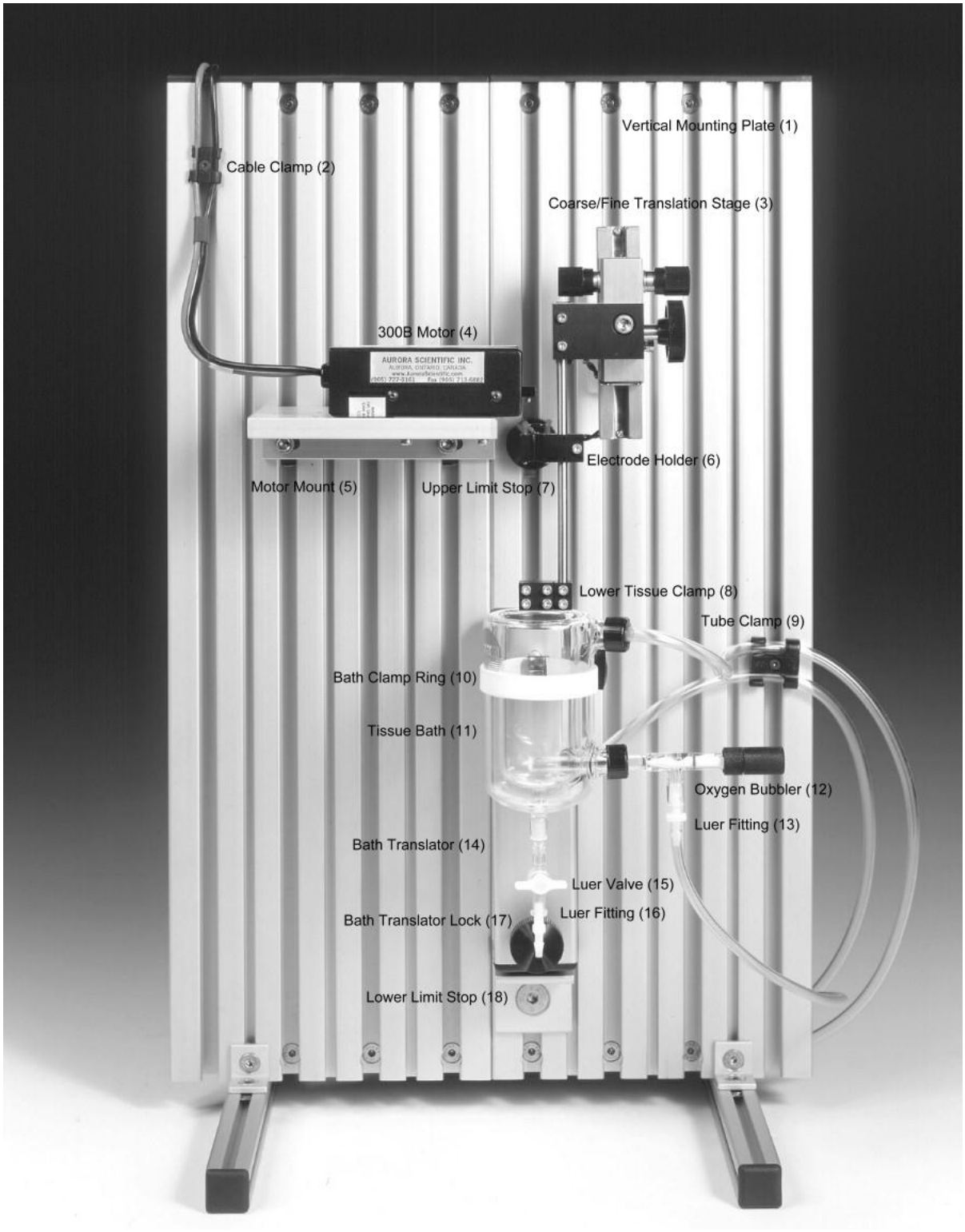


Figure 3 Annotated Photo of 800A.

2.3 Attaching the Tissue Bath

Unpack the Tissue Bath (11) and note the Bath Clamp Ring (10) around the bath and the mounting rod projecting from the back of the ring clamp. Attach the bath to the 800A/805A by inserting the ring-clamp mounting rod into the hole on the front of the Bath Translator (14). Note: there is a screw on the right side of the Bath Translator that must be loosened before the ring-clamp rod can be inserted. Don't loosen the screw too much or the captive nut located within the Bath Translator may fall out. Ensure the ring-clamp rod is firmly seated in the hole and then tighten the clamp screw. Give the clamp a slight tug to make sure that it is firmly fastened.

Loosen the Bath Translator Lock screw (17) and slowly raise the bath upwards. Ensure that the Lower Tissue Clamp (8) enters the top of the bath without touching the glass. If there is interference then check that the Bath Clamp Ring is in place properly. The position of the ring-clamp mounting rod in the bath translator may need to be re-adjusted to position the centre of the bath directly beneath the centre of the lower tissue clamp.

Ensure that you always support the Bath Translator when moving it. Under no circumstances should you allow the Bath Translator to fall unsupported. Doing this could break the bath. Tighten the Bath Translator Lock screw when not moving the stage. Caution: when both the bath and the outer jacket are filled with liquid the Tissue Bath and Bath Translator will weigh about 1 kg. Ensure that you have a good grip on the Bath Translator before loosening the Bath Translator Lock screw.

2.4 Attaching the Oxygenating Bubbler

The tissue bath comes complete with an oxygenating bubbler attachment. Insert the Oxygen Bubbler (12) in the second port up from the bottom of the tissue bath. Lock it in place by gently tightening the black lock screw. A Luer Fitting (13) is provided to allow 1/8" ID Tygon tubing to be attached to the bubbler. The bubbler includes a flow control valve. Connect an oxygen supply to the bubbler then use the valve to regulate the flow of oxygen. Ensure that an appropriate pressure regulator is attached to the oxygen supply.

2.5 Connecting the Bath Drain Valve

The bath comes with a Luer Valve (15). Simply push the Luer fitting on the valve into the outlet on the bottom of the bath. A Luer Fitting (16) is provided to allow 1/8" ID Tygon tubing to be attached to the drain valve.

2.6 Attaching the Temperature Controller to the Tissue Bath

There are two ports on the tissue bath for water to circulate through the outer jacket of the bath. Use 5/16" OD x 3/16" ID Tygon tubing to make the liquid connections. Attach the tubing to the bath using the procedure shown on the Radnoti "Quick Disconnect Instructions" page located at the end of this manual. It is recommended to connect the outlet tube from the temperature control circulator to the bottom connection on the bath and the return line to the circulator to the top outlet. This ensures any air in the system will be returned to the circulator and not get trapped in the bath. If required the bath can be rotated within the Bath Clamp

Ring by loosening the clamp screw on clamp ring and then repositioning the bath in the clamp.

A black plastic Tube Clamp (9) is provided on the right side of the vertical mounting plate. Place the inlet and outlet hoses in the clamp and then locate the clamp at a convenient location next to the tissue bath. This clamp provides support and strain relief for the water circulation hoses.

2.7 Attaching the Platinum Electrode Circuit Board

CAUTION – Turn off the stimulator and unplug it from the BNC connector on the 800A/805A before installing the electrodes.
Do not touch the stimulation electrodes when the stimulator is powered.

A circuit board with two platinum electrodes is supplied with the apparatus. The electrodes are 2mm wide x 30 AWG thick x 50 mm long (800A) or 65 mm long (805A). The platinum electrodes are soldered to the circuit board and then the circuit board is screwed to the bottom of the electrode holder (6) using two 1-72 socket head cap screws. An Allen key for these screws is provided in the tool kit. The electrode circuit board is painted to seal the circuit board and the solder holding the electrodes to the board. Research has shown that the presence of solder in the bath can allow heavy metals (lead) to leach into the bath and affect the muscle. If the black paint coating comes off of the electrode circuit board then reseal the board using rubberized paint, epoxy or nail polish.

The electrodes are attached at the factory. If the electrodes are damaged or wear out replacement electrode circuit boards can be purchased from Aurora Scientific Inc. Ensure the electrode is positioned so that the 2 mm wide surface of the electrodes face each other. This ensures that the maximum surface area faces the muscle tissue. Ensure that the electrodes are positioned close to the muscle tissue. If desired, the electrodes can be bent to decrease the distance between the electrodes and the muscle tissue. Likewise the electrodes can be cut shorter if the experimental setup requires this. The platinum can easily be cut with tin snips or even with scissors.

A red and black wire are soldered directly to the circuit board and sealed with black paint. These wires will run back to a small terminal block located near the BNC connector behind the translation stage. A small blade-type screw driver is provided in the tool kit that fits the screws on the terminal block. If the electrode circuit board needs to be replaced then loosen the terminal screws and remove the black and red wires from the terminal block. Replace the electrode circuit board and re-attach the black and red wires.

2.8 Connecting a Stimulator to the 800A/805A

CAUTION – Do not touch the stimulation electrodes when the stimulator is powered.

The electrode holder, lower tissue clamp and the upper clamp holding the support tube are all made from Delrin and are insulated from the rest of the apparatus. Do not touch the lower clamp support tube while the stimulator is operating.

A BNC connector is located on the top right side of the 800A/805A behind the Coarse/Fine Translation Stage. Use a BNC to BNC patch cable to connect the output of the stimulator to this connector.

A red and a black wire are connected to the BNC connector and these must be connected to the terminal block on the electrode circuit board.

3.0 Using the 800A/805A

3.1 Adjusting the Upper and Lower Limit Stops of the Bath Translator

The Lower Limit Stop (18) is a right-angle bracket located beneath the bath translation stage. This bracket can be repositioned to control the lower limit of motion of the Bath Translator (14). Simply loosen the screw (use a metric Allen key), slide the bracket to the new position and retighten the screw.

Likewise there is an Upper Limit Stop (7). This stop has a black plastic knob on it to allow the user to easily loosen the screw and slide the stop to a new position. Care should be taken to ensure that the stop is positioned so that the Lower Tissue Clamp (8) cannot hit either the Oxygen Bubbler (12) or the bottom of the Tissue Bath (11).

3.2 Adjusting the Lower Tissue Clamp Assembly

The vertical position of the Lower Tissue Clamp assembly can be controlled in two ways. The first is the most common method and involves using the Coarse/Fine Translation Stage (3) located at the top of the lower tissue clamp rod. Turn the large handle for coarse movement of the lower tissue clamp. The small handle provides fine adjustment of the position and can be used for setting resting tension. The stage has a small locking screw located on its side, use the small flathead screwdriver provided to loosen the screw. After setting the coarse location this lock screw should be tightened to prevent unwanted movement of the translation stage.

The second method is to reposition the entire lower tissue clamp assembly on the vertical mounting plate. Use a metric Allen key to loosen the two mounting screws and slide the entire assembly to the desired position. Ensure that you hold the entire assembly firmly when you loosen the mounting screws. If the assembly slides down in an uncontrolled manner the lower tissue clamp can break the tissue bath.

3.3 Adjusting the Location of the Lever Arm

The location of the motor and thus the lever arm are fixed by the position of the Motor Mount (5). Two adjustments are possible, the motor can be raised and lowered and the Motor Mount can be shifted a small amount to the left or right. To adjust the vertical position, loosen the two screws that fasten the Motor Mount to the Vertical Mounting Plate (1) and then slide the entire Motor Mount and Motor up or down. Retighten the screws when the mount is in the desired location.

To adjust the horizontal position of the Motor Mount loosen the two screws that fasten the Motor Mount to the Vertical Mounting Plate and then slide the Motor Mount left or right to the desired location. Retighten the screws when the adjustment is complete.

3.4 Attaching Muscle to the 800A/805A

CAUTION – to prevent electrical shock turn off the stimulator and unplug the BNC cable from the 800A/805A before attaching the muscle tissue. The stimulation electrodes will be exposed during the process of attaching the muscle.

Ensure that the data acquisition program is running on the control computer before attaching muscle tissue. The 300C/305C should be turned on and all controls adjusted correctly. This ensures that the arm will be held in the correct starting position and that force and length can be viewed on the computer screen. It is also recommended that the Length Offset potentiometer knob on the front panel of the 300C/305C be set to its middle position (5 on the turns-counting dial).

The muscle is located vertically between the lever arm and the lower tissue clamp. Before attaching the muscle ensure that the Upper Limit Stop (7) is adjusted correctly so that the Lower Tissue Clamp (8) does not touch the Oxygen Bubbler (12). Ensure that the Lower Limit Stop (18) is set so that the when the Tissue Bath (11) is in the down position the Lower Tissue Clamp (8) is fully exposed (see Figure 4).

Figure 4 Tissue Bath in Down Position. Lower Tissue Clamp is Fully Exposed.

It is recommended that before attaching the muscle the Coarse/Fine Translation Stage (3) should be adjusted close to the centre of its range of motion. Once the muscle is attached there will be the up to ±21mm of vertical range of movement available to set the resting length and tension of the muscle.

Lower End of Muscle

Loosen the screws on the front of the Lower Tissue Clamp (8) using the appropriate Allen key (do not loosen the screws that hold the clamp to the vertical stainless steel support tube). Position the muscle tissue (usually part of the tendon) between the plates of the clamp and tighten the screws.

Alternatively you could tie suture to the muscle or tendon and then clamp the suture material in the Lower Tissue Clamp. Another option is to clamp a wire hook in the Lower Tissue Clamp and then attach the muscle to the hook. Whichever method is selected, ensure that the tissue is held firmly by the Lower Tissue Clamp and that the material you use to hold the muscle doesn't stretch.

Upper End of Muscle

Suture material or wire can be attached to the upper end of the muscle (normally attached to the tendon) and then attached to the lever arm of the 300C/305C. The lever arm has a hole near the tip that can be used for a hook or simply tie the suture through the hole. When attaching the upper end of the muscle an effort should be made to minimize any slack in the attachment. An alternative method is to fashion an "S" hook from stainless steel wire and then place one end of the hook through the hole in the lever arm. Loop the suture over the other end of the hook when attaching the muscle tissue.

3.5 Measuring the Resting Length

Before raising the bath into place use a Vernier caliper or ruler to measure the resting length of the muscle tissue.

3.6 Adjusting Electrode Position

Caution: turn off the power to the stimulator and disconnect the BNC stimulator cable from the 800A/805A before touching the electrodes.

Adjust the position of the electrodes so that they are on opposite sides of the muscle tissue. Ensure that the electrodes are located along the length of the muscle and that they are positioned 0.5mm or less from the muscle. Ensure that the electrodes are not in direct contact with the muscle. Also ensure that the electrodes are not in contact with the lower tissue clamp support rod. The Electrode Holder (6) can be re-positioned to any convenient location along the length of the lower tissue support rod by loosening the lock screw in the holder and sliding the holder up or down. Re-tighten the locking screw after positioning the electrode holder.

3.7 Raise the Tissue Bath

Loosen the Bath Translator Lock knob (17) and then raise the bath into place so that the muscle is submerged. Adjust the flow of oxygen and re-attach the stimulator to the 800A/805A.

3.8 Adjusting the Resting Tension

Once the muscle is attached use the coarse knob of the Coarse/Fine Translation Stage (3) to remove any slack from the muscle. Then use the fine knob to set the resting tension. The Length Offset potentiometer knob on the front panel of the 300C/305C can also be used to move the arm up or down to obtain the desired resting tension. Normally you shouldn't need to move the Length Offset knob more than a few minor divisions either way to fine-tune the resting tension.

Figure 5 Tissue Bath in Up Position. Ready to Test.

Radnoti Instruction Sheet

Radnoti

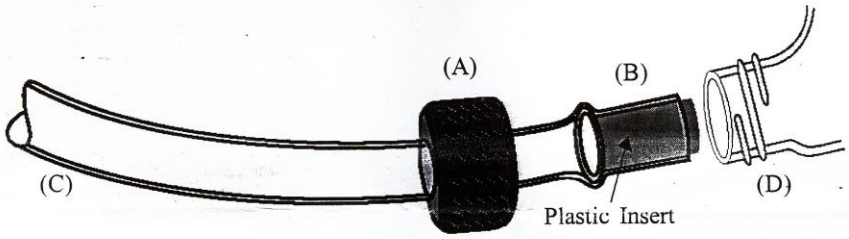
RADNOTI "QUICK DISCONNECT" INSTRUCTIONS

A. Slide CAP-WITH HOLE onto the water line (Tygon tubing 120159).

B. Push the Plastic Sleeve Insert into the end of the Tygon tubing with the flared end first. (Catalog No. 120160).

C. Be sure to use 5/16 x 3/16 inch Tygon tubing (Catalog No.120159).

D. Moisten tip of Tygon tubing before insertion into the threaded glass.
CAUTION: *To Avoid Breakage, Do Not Over-Tighten The Screw Cap.*



Replacement Part List:

Catalog No.	Description
120160	Plastic Sleeve Insert, 25/Pkg
120159	Tygon Tubing 5/16 x 3/16 ID, 50 Ft/Min.
160196	Drilled caps for Water Jacketed QD Connections 12/Pkg

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