

INSTRUCTION MANUAL

Model 809B

***In situ* Test Apparatus for Muscle Lever Systems**

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1.0 Introduction

The 809B *in situ/in vitro* test apparatus is used with a 300C/300C-LR/300C-FP muscle lever system, but can accommodate a 305C/305C-LR/305C-FP if necessary. The apparatus enables physiology researchers to easily test muscle tissue *in situ*, perform footplate style experiments or test isolated muscle *in vitro* with an Aurora Scientific Inc. (ASI) muscle lever system. The apparatus was primarily designed for use with mice however it can accommodate other small animals of approximately the same size. The 809B consists of a heated anodized aluminum mounting plate on which the animal lies and the limbs are affixed, various clamps for immobilizing the limb, motor mount and a dual mode coarse/fine horizontal translation stage for adjusting resting tension or resting length. Also included are screws to mount the motor to the motor mounting plate extra screws that allow the height of the limb plate to be adjusted and a set of metric Allen keys. An *in-vitro* bath plate for isolated muscle experiments is optional. The animal plate can be temperature controlled by circulating water through the plate. A lab circulating bath can easily maintain the animal plate at the desired physiological temperature. The 809B frame is designed to allow the animal plate to easily be moved with respect to the motor. This allows the system to be adjusted to suit the size of the animal and the desired attachment point on the animal. The animal plate can be locked at the desired horizontal location with thumb screws. Likewise the vertical and lateral position of the motor can also be adjusted through a wide range with fine control due to the motor being mounted on a dual axis precision stage.

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

The location of the motor with respect to the tissue can be fine adjusted by using the fine horizontal translation stage connected between the motor clamp and the motor mounting plate. The fine position control allows the resting tension or resting length of the muscle tissue to be easily and accurately set. Once positioned the fine translation stage can be locked in place with the knob located on the side of the stage.

The height of the animal plate can be adjusted by removing or adding spacers. The lateral position of the limb plate can also be adjusted to two separate locations. A series of tapped mounting holes, in the limb plate, allow the limb clamps to be positioned over a wide range. In addition to the supplied limb clamps, the limb plate has been drilled with 1.0 mm diameter holes that can accept pins that can be used to hold the limb in place.

1.1 Specifications

Frame:	Anodized extruded aluminum “T” profile
Overall Dimensions:	305 mm L x 220 mm W x 140 mm H
Animal Plate:	Anodized Aluminum
Dimensions:	115 mm L x 90 mm W
Water Fitting:	1/4” Hose ID dry-break quick-connect fitting
Water Fitting:	1/4” push lock
Movement:	100 mm
Mounting Holes:	29 – M3 tapped holes on 12.7 mm (0.5”) centers
Pin Holes:	25 – 1.0 mm diameter holes on 12.7 mm (0.5”) centers
Clamping Fixtures:	Limb clamp with conical fixating screws Limb clamp for 1.0mm drill bits Knee clamp with sharpened fixating screws Knee clamp with buttressing Delrin pad.
Motor Movement	
Vertical:	40 mm
Horizontal:	100mm
Temperature Control:	Animal plate has water channels to provide stable temperature control. Dry-break, quick-connect fittings supplied.
Accessories:	M6x20mm, M6x40mm, M6x65mm stainless steel socket head cap screws for adjusting platform height. 10mm, 15mm and 20mm spacers for adjusting platform height. M3x6mm stainless steel socket head cap screws for mounting the 300C motor. M4x10 mm stainless steel socket head cap screws for mounting the 305C motor. Metric Allen key set. Water circulation tubing (2 6-foot lengths), and dry-break quick-connect fittings for the tubing.

2.0 Apparatus Setup

2.1 Unpacking

Unpack the apparatus from the shipping container. The 809B comes completely assembled. Screws are included to mount the 300C/300C-LR motor to the motor mount. Also enclosed are a set of metric Allen keys, the motor mount screws, the drill clamp, spacers and extra screws. In the main shipping container are 2 6-foot lengths of Tygon tubing complete with quick-connect fittings. Remove all parts from the shipping container.

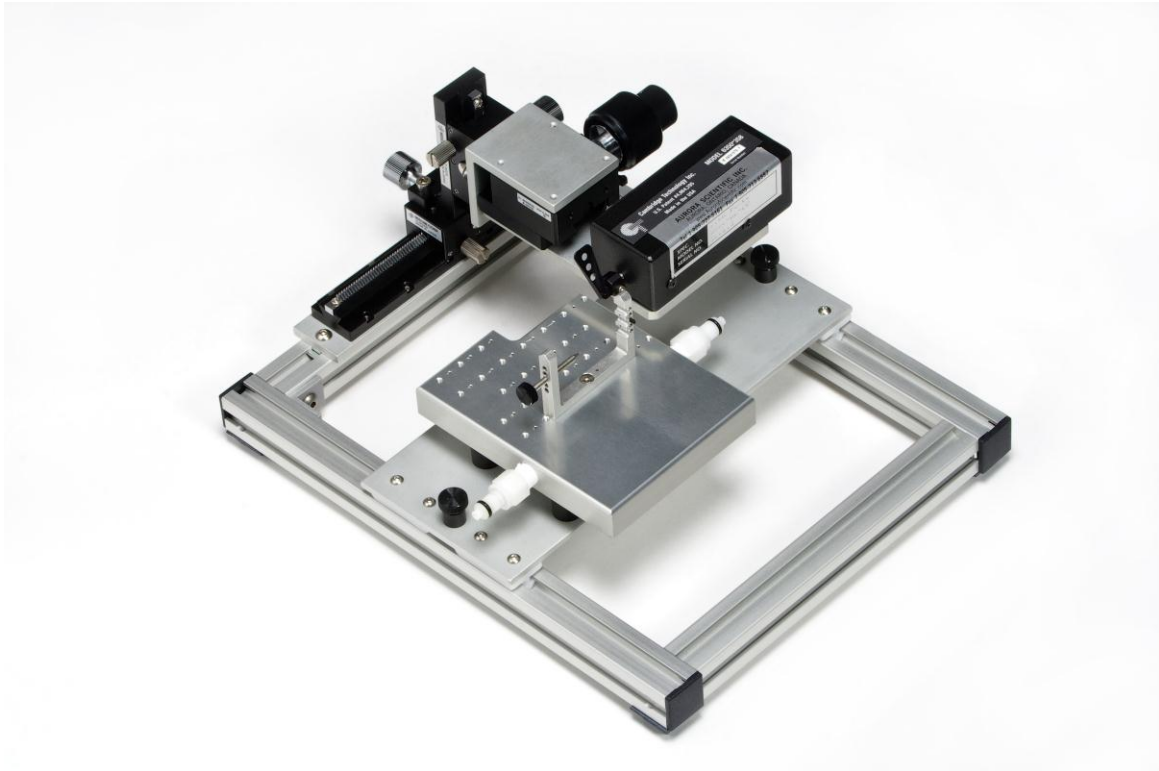


Photo 1 809B with 300C Motor Attached

2.2 Attaching the Motor

Refer to drawing 809A-A001 at the back of this manual and mount the motor (4) on the Motor Mount (10) with the shaft of the motor pointing towards the left side of the apparatus and the lever arm pointing down, or footplate pointing up, also see Photo 1. Remove the four motor mount screws from the package (the 300C mounts with M3x6mm button head cap screws, the 305C mounts with M4x10mm socket head cap screws,) and attach the motor to the plate using the metric Allen keys provided.

The lever arm or footplate (20) can be attached before or after the motor is mounted.

Position the motor cable so that it does not get pinched when the animal plate is moved or when the motor is moved either up and down or laterally.

2.3 Attaching the Temperature Controller to the Animal Plate

There are two fluid connectors (30) on the side of the animal plate (5). Two 6-foot lengths of 1/4" ID x 3/8" OD vinyl tubing have been supplied with the mating quick-connectors attached to the tubing. These tubes should be attached to a temperature controlled lab circulator. One connector attached to the supply and the other attached to the return. It doesn't really matter which connector is attached to the supply however the side of the animal plate that is attached to the supply side of the circulator is slightly warmer than the return side. If the animal is positioned on one side of the animal plate it may be best to attach the supply to that side.

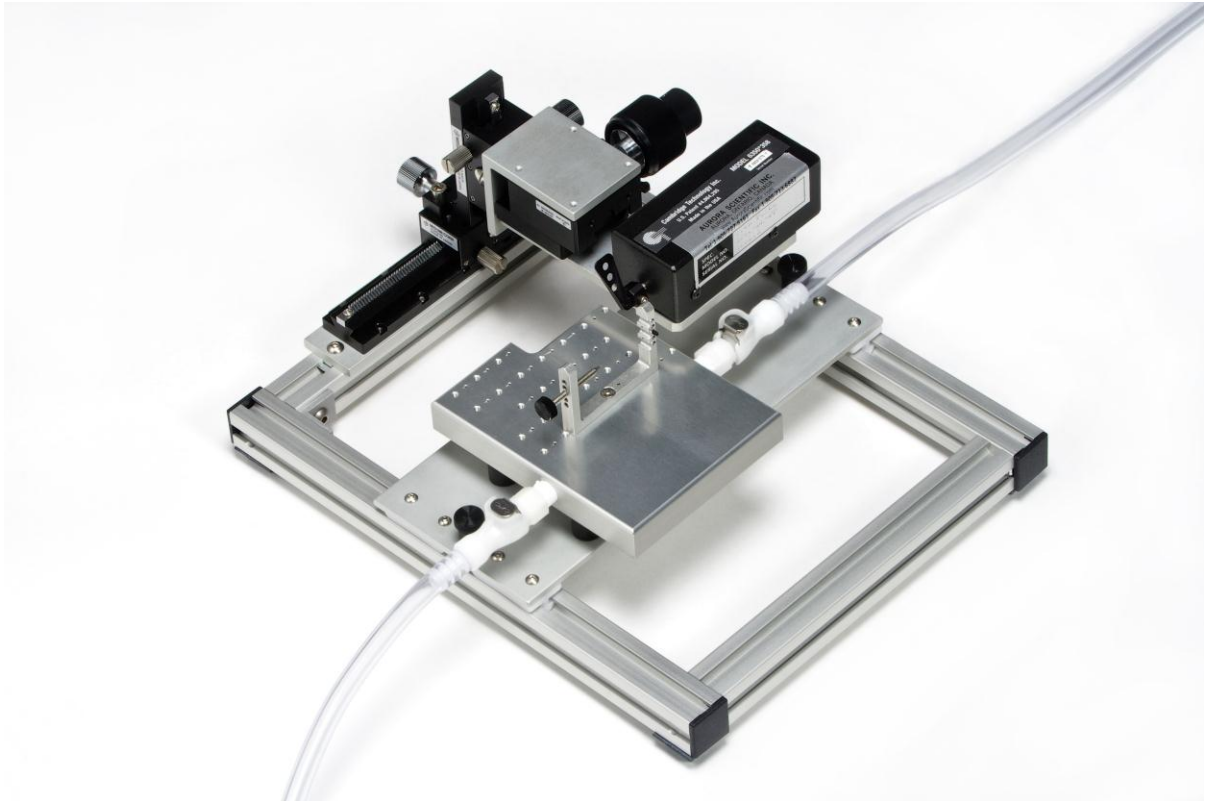


Photo 2 809B with Water Lines Attached

3.0 Using the 809B – Animal on its side/Animal on its back

3.1 Adjusting the Position of the Animal Plate

The animal plate (5) is an aluminum plate with a water channel machined inside it. There are 2 locking knobs (31) that lock the plate in place on the frame. Loosen these knobs about 1 turn and then slide the animal plate to the desired position. It is convenient to move the animal plate as far away from the motor as possible while attaching the animal and performing surgery. Once the animal is ready to have the lever arm attached simply slide the animal plate back towards the motor and lock it in the desired position. Fine control of the motor/lever arm position, with respect to the animal, is accomplished using the translation stage directly behind the motor (27) (see section 3.5 for further details of fine positioning).

3.2 Attaching the Animal to the 809B

Muscle mechanics experiments that are done *in situ* often involve resting the anesthetized animal on a temperature controlled platform and then clamping the limb of interest. The animal plate provides a large surface for resting the animal. If necessary the animal can be held in place using tape, Velcro or other attachment means. If tape is used it is best to avoid having the tape wrap over the aluminum frame as this will prevent movement of the animal plate.

The limb can be held in place using any or all of the clamps provided. Three clamps are included with the 809B. The first clamp (11) features a single conical fixating screw with a mating conical set screw that is screwed into the limb platform (see Photo 3). The second clamp has two vertical slots and a clamp plate that allow a 1.0 mm drill bit to be clamped vertically between one of the 1.0 mm holes in the limb plate and the clamp (see Photo 4). The third clamp is strictly for footplate experiments and is U shaped. The knee of the animal is immobilized either by using sharpened screws which fasten into the clamp horizontally or by buttressing the knee against a channel in the side of the clamp. Either of the clamps can be positioned at any desired position on the grid of M3 tapped holes located on the animal plate. Note that all of the clamps have a 1.0 mm dowel pin protruding from their base. This dowel pin must be inserted into one of the mating holes drilled in the limb platform. Simply remove the screw holding the clamp in place, lift the clamp vertically off of the limb platform and reposition it at the desired location.

The conical fixating screw clamp is used by clamping the bone between the tips of the upper and lower screw. The upper screw is then tightened to keep the bone motionless. The drill bit clamp is used to clamp a 1.0 mm drill bit that has been drilled through the bone and left in place in the bone. The lower end of the drill bit is slid into one of the 1.0 mm holes in the limb platform and then upper end of the drill bit is held by the clamp. A small knob is included to allow the clamp plate to be tightened over the drill once it is in place.

The height of the Limb platform can be adjusted by choosing a combination of the black Delrin spacers that support the Limb plate. Simply remove the four screws holding the animal plate to the aluminum base plate then select the desired spacers and reinstall the screws. Different length M6 socket head cap screws are supplied to provide screw lengths appropriate to all heights. Note that there are small nylon shoulder washers installed in the top and bottom of each hole on the limb plate. These spacers should always be in place as they provide thermal insulation between the screws and the heated limb platform.

The lateral position of the Limb plate can also be adjusted 25.4 mm to the left of the centerline by mounting the Limb plate in the other tapped holes on the aluminum base plate.

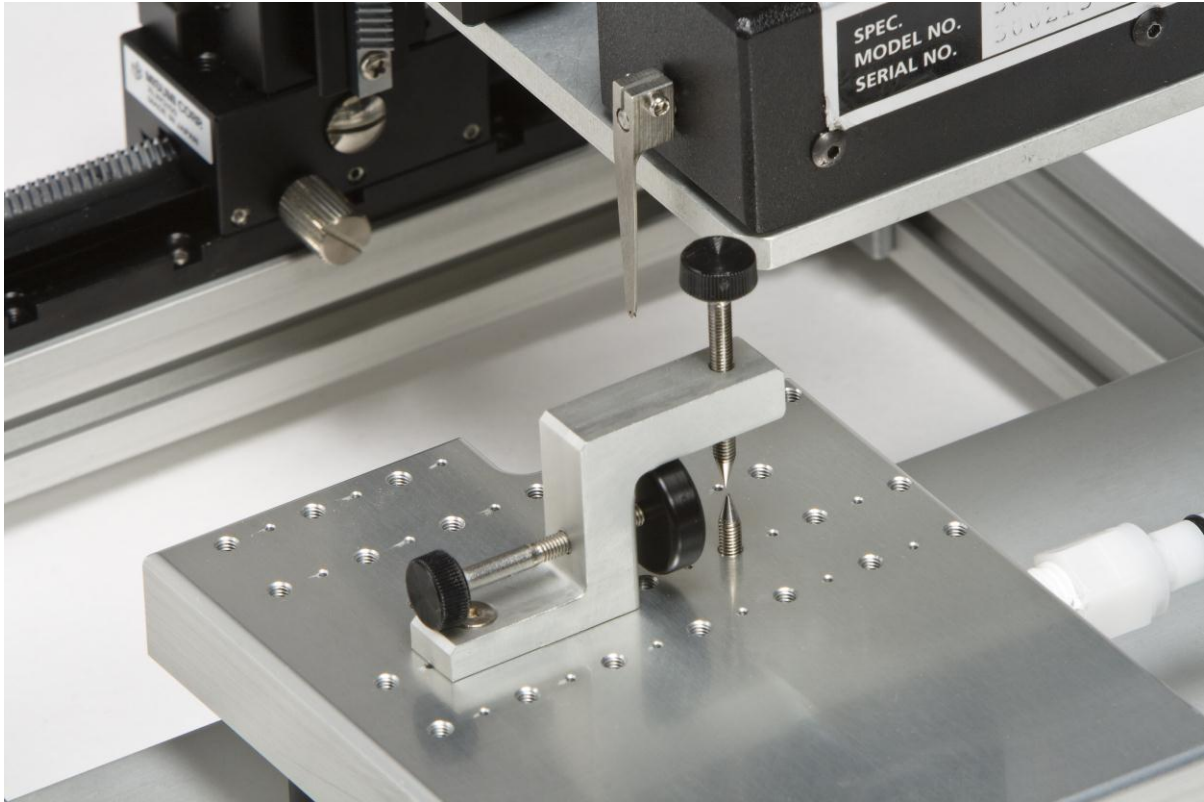


Photo 3 Limb Plate with Screw Clamp

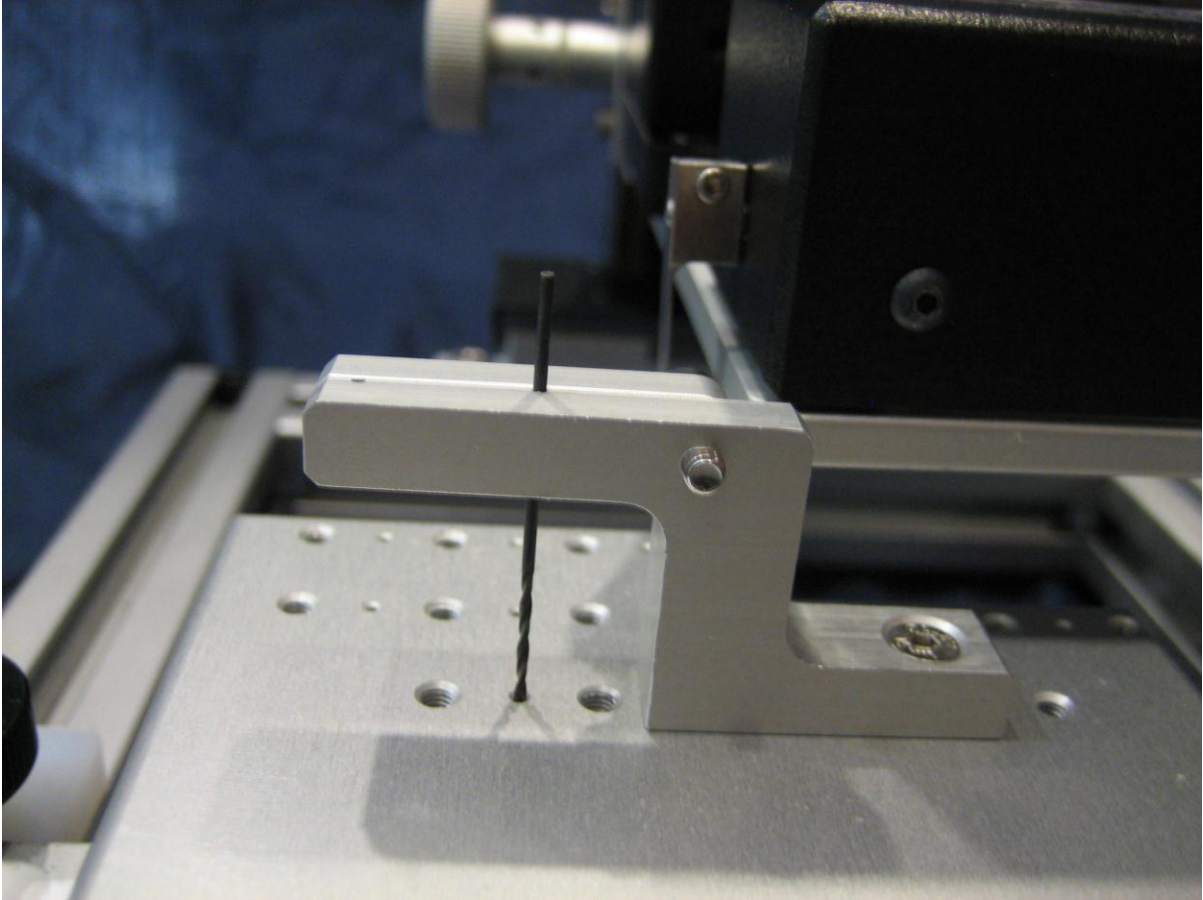


Photo 4 Limb Plate with Vertical Drill Clamp

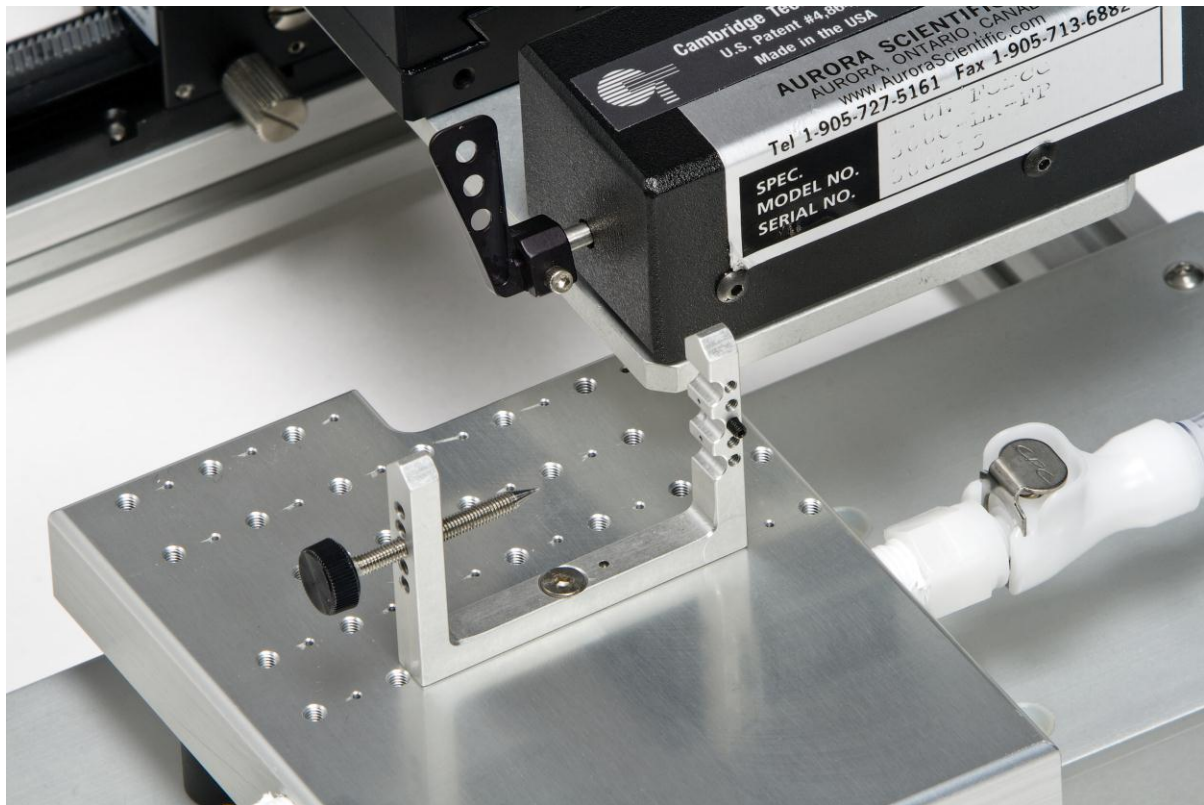


Photo 5 Limb Plate with Knee Clamp and footplate

3.3 Adjusting the Motor Position

The motor will normally need to be positioned prior to attachment of the muscle or tendon to the lever arm. There are three controls that can be used to position the motor with respect to the animal's limb. To adjust either the horizontal or vertical position of the motor, simply unlock the stages locking knobs and manipulate the position knob to the desired position.

It is best to support the motor frame with your hand when unlocking the vertical translation locking screws. It is also wise to move the animal plate as far forward as possible (away from the motor) prior to adjusting the motor's vertical position. This ensures that the motor and lever arm will not be above the Limb plate or the aluminum base plate and if the motor frame is accidentally allowed to drop the lever arm will not hit anything. Once the motor height has been adjusted, slide the animal plate back into position.

The position of the motor can be fine adjusted using the fine adjustment translation stage. This stage provides 1.0 mm of movement per rotation and a total of +/-10. mm of movement. This stage is used to set the resting tension as described in section 3.5.

3.4 Attaching the Foot to the Footplate

As is the case when attaching a muscle directly to the lever arm the data acquisition program and controls for the motor should be properly adjusted. Additionally both the offset potentiometer knob and the offset of the Fine Translation stage should be set at their center positions.

The foot of the animal can be secured in the footplate by wrapping a small amount of tape around the plate itself to keep the foot from slipping out. Once the foot has been secured in the footplate and there is no slipping or movement within the footplate itself, the knee of the animal should then be clamped at a desired height and distance from the plate itself.

For general instructions on how to tie on to the muscle during an in-situ experiment please see section 4.3.

3.5 Adjusting the Resting Tension

A Fine Translation Stage (Photo 5) is provided to allow the resting length or resting tension to be set. Each rotation of the knob moves the motor 1.0 mm. A locking screw is provided on the side of the translation stage to lock the stage in position. It is recommended to tighten this screw prior to taking data.

Another method of fine adjusting the resting tension is to use the Length Offset potentiometer knob on the front panel of the 300C. This control will move the lever arm back and forth to obtain the desired resting tension. Normally the resting tension is set with the translation stage and then fine tuned with the Length Offset control. You shouldn't need to move the Length Offset knob more than a few minor divisions either way to fine-tune the resting tension.

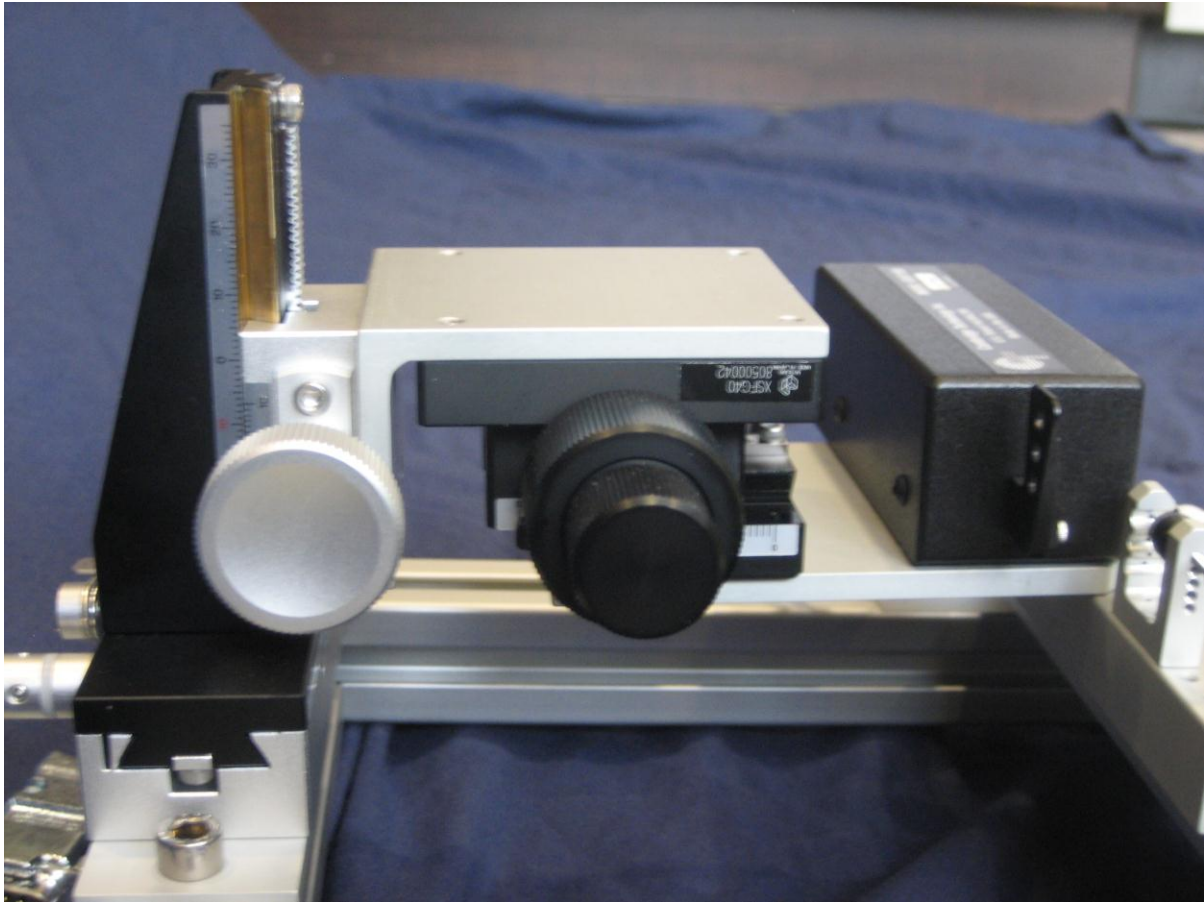


Photo 6 Close-up of Motor Fine Position Stage

4.0 Using the 809B-IV – Isolated Muscle in a bath

4.1 Adjusting the Position of the Bath Plate

The bath plate is an aluminum plate with two water channel machined inside it. There are 2 locking knobs (31) that lock the mounting plate in place on the frame. Loosen these knobs about 1 turn and then slide the animal plate to the desired position. Once the muscle is surgically ready and removed it may be placed in the bath to be sutured to the tissue post provided. Fine control of the motor/lever arm position, with respect to the animal, is accomplished using the translation stage directly behind the motor (27). (See Section 3.5 for fine positioning from the motor arm end.)

4.2 Setting up the 809B-IV Chamber

Muscle mechanics experiments that are done *in vitro* will involve some sort of chamber with a Ringer's/Krebs type solution. The In-Vitro chamber has 25mL of volume for the desired solution, and has water lines which can maintain the bath temperature at a desired set point. Other extras included a thermocouple holder for recording temperature or providing feedback to a water bath, Supply and Return Lines for Oxygen Perfused Media, as well as a fine micromanipulator for tissue post positioning.

As with the animal platform, there are two fluid connectors on the rear of the bath plate. Two 6-foot lengths of 1/4" ID x 3/8" OD vinyl tubing have been supplied with the mating quick-connectors attached to the tubing. These tubes should be attached to a temperature controlled lab circulator. One connector attached to the supply and the other attached to the return. It doesn't really matter which connector is attached to the supply however the supply side of the bath will be slightly warmer than the return side.

A BNC cable should also be used to connect to the male BNC post on the rear of the bath plate to provide stimulation pulses from either an ASI 700 Series or client supplied stimulator. See photo 7 for a picture of the fully connected setup in its In-vitro configuration.

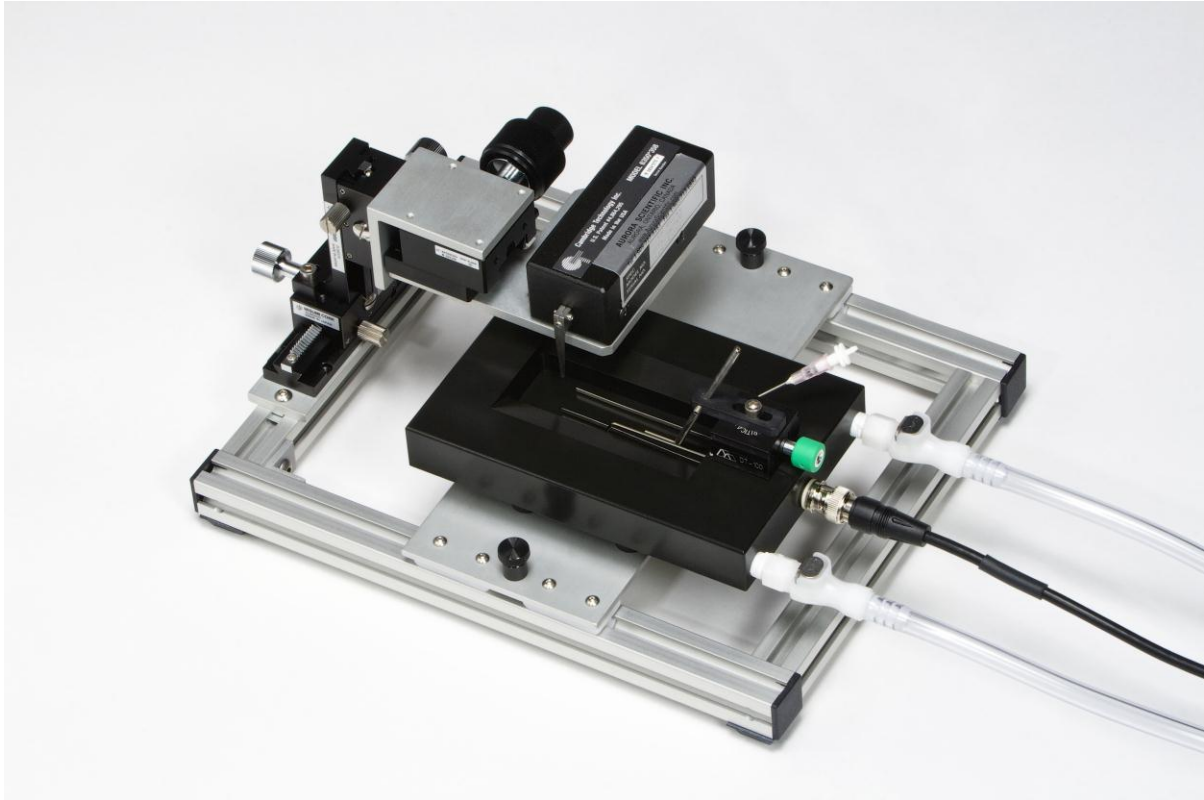


Photo 7 Close-up of Motor Fine Position Stage

4.3 Attachment of the muscle

Ensure that the data acquisition program is running on the control computer before attaching muscle tissue. The 300C 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 be set to its middle position (5 on the turns-counting dial or 5 turns from either end on older models that don't feature a turns counting dial).

It is recommended that before attaching the muscle the Fine Translation Stage should be adjusted close to the centre of its range of motion. Once the muscle is attached then there will be the up to ± 10 . mm of horizontal range of movement available to set the resting length and resting tension of the muscle.

Suture material or wire can be attached to the muscle (normally attached to the tendon) and then attached to the lever arm of the 300C. 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 muscle it is not that important to minimize slack in the attachment since this slack can be taken up by a combination of moving the animal plate and moving the translation stage. However the attachment method should offer little extra compliance. One method of attaching the suture to the arm 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.

To suture to the tissue post when using isolated muscle and the in-vitro bath, the tendon of the muscle should be tied to the bottom part of the hook securely with silk suture. If silk suture cannot be sourced then dental floss is an acceptable alternative. The resting tension can then be set in the same manner as in chapter 3.5.

5.0 Terms and Conditions for Returning Equipment

1. Aurora Scientific Inc. **will not** accept any equipment returned without prior authorization in the form of a return material authorization number.
2. **Please call Customer Service at (905) 727-5161 or toll free at 1-877-878-4784 to obtain an RMA number. Please specify the product line.**
3. Please package equipment properly. Goods that are damaged in shipment are the responsibility of the shipper.
4. **Aurora Scientific, Inc. withholds the right to assess charges for the repair or replacement of such damaged goods, regardless of warranty status.**
5. Warranty repairs will be shipped back to the customer via FedEx. If you require or request another form of shipment, the cost of such service is your full responsibility.
6. Aurora Scientific, Inc. **will not** be responsible for any return or replacement **shipping charges** incurred due to an incorrect order placed by the customer.

Return Shipping Address:

Aurora Scientific Inc.
360 Industrial Pkwy. S., Unit 4
Aurora, ON, Canada
L4G 3V7
Attn: RMA Returns

6.0 Warranty

The 809B *in situ* Apparatus is warranted to be free of defects in materials and workmanship for three years from the date of shipment. Aurora Scientific Inc. will repair or replace, at our option, any part of the system that upon our examination is found to be defective while under warranty. Obligations under this warranty are limited to repair or replacement of the instrument. Aurora Scientific Inc. shall not be liable for any other damages of any kind, including consequential damages, personal injury, or the like. Disassembly of the unit will void this warranty. Damage to the system through misuse will void this warranty. Aurora Scientific Inc. pursues a policy of continual product development and improvement therefore we reserve the right to change published specifications without prior notice.

Drawings

This section consists of the following drawings:

- 1) 809A in situ Test Apparatus 809A-A001, Rev. 0