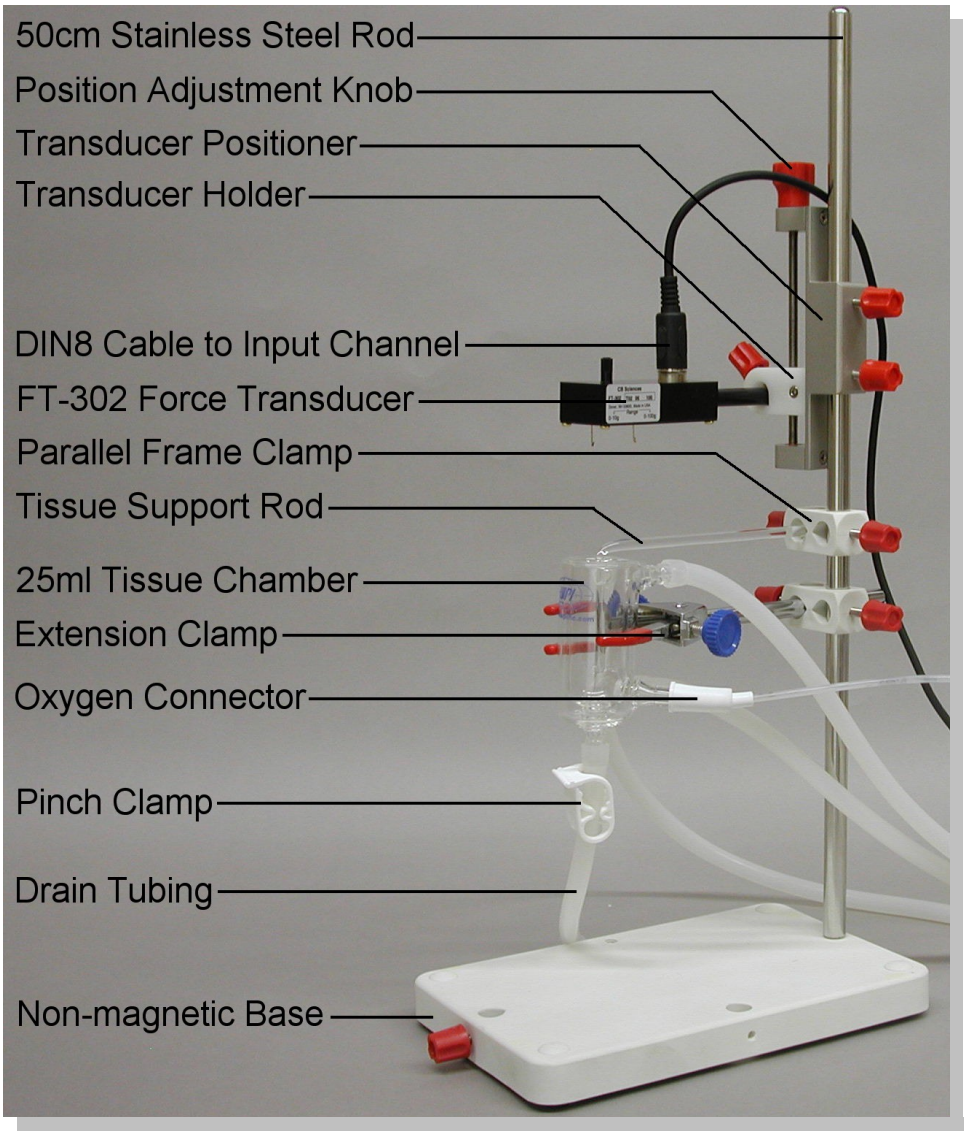
STB-125 Student Tissue Bath

Technical Note



STB-125 with FT-302 Force Transducer

Overview

The STB-125 is a single channel tissue bath assembly with many features that make it suitable for use in both teaching and research laboratories. Experiments on a variety of skeletal, smooth, and cardiovascular muscle tissues can easily be performed with the STB-125.

The 25 ml tissue chamber in the STB-125 is designed to accommodate tissue segments of different lengths, provide ample space for the placement of the tissue in the chamber, and minimize the volume of buffer needed to fill the chamber. The chamber is fitted with a glass bubbling frit for proper aeration of the tissue and buffer. The temperature of the tissue and buffer in the chamber can be maintained by circulating water through the jacket that surrounds the tissue chamber.

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STB-125 Student Tissue Bath

The STB-125 includes a transducer positioner for setting the length and tension of the tissue through the ease of turning a knob that raises or lowers the transducer. The separate glass tissue support rod in the setup makes it easy to position the tissue in the chamber and to hold the length and tension of the tissue as the chamber is adjusted.

All of the items pictured in the labeled photograph of the STB-125, except the force transducer, are included in the STB-125 student tissue bath. The components included in the kit are listed in the table below.

Components of the STB-125 Student Tissue Bath		
Quantity	**Part Number**	**Part Description**
1	A-TB-NMB-125	Non-magnetic base
1	A-TB-BATH-125	25ml Tissue bath
2	A-TB-XBLK	Brass parallel frame clamp
1	A-TB-GEC-125	Glassware extension clamp
5 ft	A-TB-ST15-125	Silicone tubing, 0.153” OD X 0.106” ID
1	A-TB-O2C-125	Oxygen connector
10 ft	A-TB-ST25-125	Silicone tubing, 0.250” OD X 0.438” ID
1	A-TB-PPC-125	Polypropylene pinch clamp
2	A-TB-FHFL-125	Flat loop tissue clips
2	A-TB-FLC-125	Fine loop tissue clips
1	A-TB-GTS-125	Glass tissue support
1	A-TB-MXBLK	Transducer positioner

How to Use the STB-125

Assembly

1) Use the components listed in the Components table to assemble the STB-125 Student Tissue Bath.
2) Thread the 50 cm stainless steel rod into the matching threaded hole on the non-magnetic base (A-TB-NMB-125). With the rod in this position, the base will provide a stable support for the components aligned above it.
3) Place a brass parallel frame clamp (A-TB-XBLK) on the stainless steel rod. This clamp should be about 20 cm above the surface of the base and aligned as seen in the photograph.
4) Place the arm of the glassware extension clamp (A-TB-GEC-125) in the clamping hole on the side of the parallel frame clamp. Position the extension clamp so its prongs can hold the tissue chamber vertically over the front half of the base.

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5) Place a second parallel frame clamp (A-TB-XBLK) on the stainless steel rod. Align this clamp above the first clamp. The second clamp will eventually hold the glass tissue support (A-TB-GTS-125) that will hold the lower end of the tissue in the chamber.

Do not put the glass tissue support on the second plastic parallel clamp until the tissue is ready to be mounted in the chamber. The final position of the second parallel frame clamp will be a couple of centimeters above the first parallel frame clamp.

6) Place the transducer positioner (A-TB-MXBLK) on the stainless rod with the adjustment knob of the positioner on top. Clamp the positioner on the rod so the bottom of the positioner is about 30 cm above the base.
7) Clamp the appropriate transducer in the holder on the threaded rod of the positioner. Align the positioner and the transducer so that the hole or hook on the arm of the transducer is directly over the center of the tissue bath chamber.
8) Turn the adjustment knob on the positioner to move the transducer and its holder to the middle of the threaded rod.
9) Determine the best location on the lab bench for the tissue bath setup. The setup should be convenient to a sink or a drain flask, a mixed gas supply used for aeration, a water bath used for warming flasks of buffer used in the experiment, a warm water supply or a heating circulator used to maintain the temperature of the tissue and the buffer, and the data acquisition system used to record the responses of the tissue during the experiment.

Warm buffer can be stored in an optional 1L buffer reservoir (A-TB-RES-125) that can feed buffer directly into the tissue chamber. The oxygen saturation level of the buffer in the reservoir can be maintained with a matching bubbler connected to the aeration system. The reservoir can be held on a ring stand or rack with a matching clamp. The temperature of the tissue and buffer can be maintained by connecting an optional heating circulator (A-TB-HC), with a working temperature range from 25-100°C, to the water jackets of the tissue chamber and the buffer reservoir.

10) Find the coils of silicone (A-TB-ST25-125) and (A-TB-ST15-125) tubing.
11) Cut the A-TB-ST25-125 silicone tubing into the lengths needed to make a drain line, an overflow line or a supply line to a buffer reservoir, and water lines to and from a warm water supply needed to maintain the temperature of the tissue bath.
12) Feed about 8 cm of the drain line through the two circular holes on the plastic pinch clamp (A-TB-PPC-125). Carefully put the end of the drain line with the pinch clamp on the drain port of the tissue bath. Leave the drain open.

13) Carefully put the ends of the additional fluid lines on the appropriate ports of the tissue bath.
14) Put the oxygen connector (A-TB-O2C-125) on the aeration port of the tissue bath.
15) Carefully clamp the assembled tissue bath between the prongs of the glassware extension clamp already on the stand.
16) Attach one end of the silicone tubing (A-TB-ST15-125) to the oxygen connector on the tissue chamber. Connect the other end of the silicone tubing to the valve on the cylinder containing 95% O2 and 5% CO2.
17) Place the end of the drain line in the sink or a flask used to collect waste buffer. Connect the tubing on the water inlet, at the bottom of the tissue bath, to the warm water supply at the sink or to the outlet of a heating circulator. Place the end of the tubing on the water outlet, at the top of the tissue bath, in the sink, or on the inlet of the heating circulator.

Calibrate the Transducer

1) Make sure that the iWorx data acquisition system is turned on and the transducer is connected to the system for a ten-minute warm-up period before the calibration is done.
2) Follow the calibration instructions for the FT-302.

Placement of Tissue in Chamber

1) Use a clean beaker to obtain about 100 ml of buffer solution. Reserve this beaker for transferring clean buffer throughout the exercise.

Avoid contamination! Take only as much buffer as needed for each rinse or buffer change. Do not return any buffer taken from the supply flask back to the supply flask!

2) Rinse the tissue chamber thoroughly, three or four times, with buffer.
3) Close the drain of the tissue chamber and fill the chamber with about 20 ml of buffer. Open the valve on the aeration line and adjust the flow of the oxygen/carbon dioxide mixture through the aeration frit to create a plume of small bubbles.
4) Obtain a piece of tissue to use in the experiment. Keep the tissue in a beaker or dish of buffer at the desired temperature until you are ready to attach it to the support rod.
5) Work quickly and carefully when mounting the tissue in the chamber. Attach one end of the tissue to the glass tissue support using a loop of suture thread securely tied to the tissue and looped under the hook of the tissue support rod. Securely tie a piece of suture to the other end of the tissue. Make sure the suture is long enough to connect the tissue to the transducer. Tissue clips (A-TB-FHFL-125, A-TB-FLC-125) can also be used to attach the tissue to suture threads on the hooks of the tissue support and transducer. Note that

clips may slip off the tissue if the force developed by the tissue is greater than the grip strength of the clips.

6) Once the lower end of the tissue is attached to the hook of the tissue support rod (A-TB-GTS-125), lower the tissue and its support rod into the tissue chamber. Keep tension on the upper suture thread as the assembly is lowered into the chamber. This will prevent the tissue from coming off the hook on the support rod.
7) Attach the suture thread on the upper end of the tissue to the appropriate hook or hole on the arm of the transducer. The length of the tissue should be no greater than the *in situ* length.
8) Align the transducer, the tissue bath, and the tissue support rod. The suture and the tissue should be vertical, and the tissue should not be touching the inside of the tissue bath.
9) Check the temperature of the tissue bath. Monitor the temperatures of the tissue bath and water bath holding the flasks of fresh buffer.
10) Record the tension in the tissue. Observe the position of the trace on the recording as the transducer is raised by turning the adjustment knob on the positioner. Turn the knob until the trace on the screen visibly moves from its initial level. The amount of adjustment required depends on the initial slack in the tissue and in the threads holding the tissue.
11) If necessary, adjust the flow of bubbles from the aeration frit to prevent the tissue from being moved around by the bubbles.
12) Perform experiments according to directions provided in the lab protocol.

If contractions in the tissue are visible, but do not produce a noticeable movement of the recording trace, check the tension of the suture threads holding the tissue in place and check the operation of the transducer and the recording.

Experiments

LabScribe experiments that can be performed with the STB-125 Student Tissue Bath include:

- **Experiment AM-5: Intestinal Motility**, found in the **Animal Muscle** category of the LabScribe **Settings** menu as **IntestinalMotility**
- **Experiment AM-4: Uterine Motility**, found in the **Animal Muscle** category of the LabScribe **Settings** menu as **UterineMotility**

Care of the STB-125

1) After the experiment is concluded and the tissue is removed from the chamber, rinse the tissue chamber, the tissue support rod, and the drain tubing with de-ionized water.

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2) To clean the bubbling frit, fill the tissue chamber with deionized water and vigorously bubble air through the frit for a few minutes. Drain the water and repeat this cleaning procedure a couple of more times.
3) Clean any saline solutions or buffers that may have spilled or sprayed on the metal or plastic parts of the stand, the transducer and its positioner, and the clamps.