



CAMPDEN INSTRUMENTS LIMITED

INSTRUCTION MANUAL

FOR

HA752 VIBROSLICE TISSUE CUTTER
(Manual tissue transport)

MA752 VIBROSLICE TISSUE CUTTER
(Motorised tissue transport)

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The information provided in this document is intended to be a guide on how to use the Vibroslice instrument safely and effectively and is subject to change without notice. Campden Instruments does not accept liability for any damages arising from this document or the information contained in it. Campden Instruments will not accept liability for damages arising out of misuse of this instrument.

Read and understand this instruction book before using the instrument. Only competent and capable personnel should use the instrument.

This document should be retained for future reference as it contains the name and address of the manufacturer within the EC.

PACKAGING

Please retain original packaging for future use.

Instruments will not be accepted for service or repair unless the unit is returned adequately and properly packaged.

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INTRODUCTION.

The Campden Instruments Vibroslice was designed to cut 50-700µm thick slices of fresh brain tissue, but it has proved useful with a wide range of other tissues such as kidney or liver. It is also useful with fixed tissue where sections down to 20µm thick help with the penetration of reagents for histochemistry.

Vibrating blade slicers such as the Vibroslice appear to cause less damage than some alternative methods such as tissue choppers, this has proved valuable in preserving neurones in certain brain regions.

As the Vibroslice contains exposed moving parts and by its nature a blade capable of cutting tissue it is important adequate safety precautions are taken.

This instrument contains moving parts. Care must be taken not to interfere with their motion.

The blades used with this instrument are extremely sharp and should be handled with care. Dispose of used blades carefully.

Observe manufacturers guidelines when handling chemicals.

Spillage - If the cutting lubricant/preserving liquid, e.g. physiological saline, is spilt over the instrument it is important for electrical safety reasons to ensure that the instrument remains safe to use. To avoid the possibility of electrical shock if a spillage occurs, the unit should be switched off at the mains electrical outlet and disconnected before touching the instrument. The instrument should be inspected and tested if necessary by a suitably qualified technician before it is put into further use.

This instrument must not be operated unless it is adequately earthed (grounded).

All electrical instruments and equipment should be periodically tested to ensure they remain safe to use. In some countries this may be a statutory requirement. Your local Health and Safety Executive (or equivalent) will be able to advise on this matter.

User servicing is limited to changing the drive belts when necessary. The instrument contains no other user-serviceable parts. Contact your dealer or Campden Instruments if you require assistance.

Please Note:

Campden Instruments offers a service and repair facility for its instruments but before returning any equipment for service/repair please contact Campden Instruments for a Decontamination Note>Returns Authority. **Campden Instruments will not accept any equipment for service or repair unless it has been cleaned and certified free of all contaminants prior to despatch and has been adequately packaged.**

Blades

Whatever features and control an oscillating microtome possesses, the quality of the slice can be enhanced by good blades and degraded by poor blades.

The average razorblade consists of a triple bevel on both faces on a thin foil of either carbon or stainless steel. The triple bevel terminates in a relatively non-acute angle and is designed for cutting when being drawn across a surface perpendicular to the edge of the blade. The very thin foil will almost certainly flex when clamped into a blade holder. For these reasons razorblades are not recommended for tissue slicers.

Carbon steel is relatively hard compared to stainless steel and thus will keep its cutting edge longer however it also has the inherent disadvantage in that it will rust quickly when exposed to moist air. To prevent rusting in storage, all carbon steel blades have a film of oil that first needs to be removed, before the blade can be used. The corrosion process is, of course, accelerated significantly in a.c.s.f. saline.

For these reasons normal razor blades and carbon steel are not recommended for precision tissue sectioning.

Campden Instruments supplies two types of blade for all its oscillating microtome models. These are as follows:

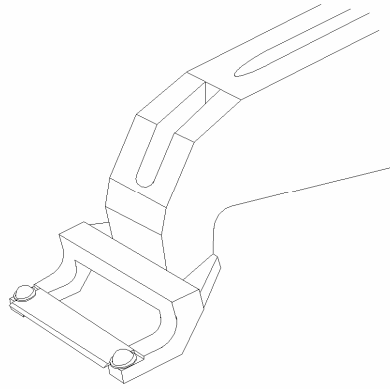
Model 7550/1/SS Stainless Steel Blades. Made from surgical quality stainless steel these are double bevelled on both faces, honed to an acute cutting edge. Because of the relative softness of stainless steel, for optimum performance, it is recommended that these blades should be used once only or at maximum changed every day.

Model 7550/1/C Ceramic Blades. Made from ultra hard zirconium, this is a material that can be honed to the finest of edges, the body of the blade can also be thinner than a metal blade whilst still remaining rigid and hence maintaining a straight cutting edge. The result, definitively, is that slice quality is substantially improved with prolonged slice life especially in the most difficult of tissues such as young brain where structures are yet to be formed or very old brain with build up of extracellular proteins and added structures. Additionally, the blade has a much longer life due to the ultra hard cutting edge not losing its sharpness and being impervious to corrosion.

For studies where the deposition of metal into the slice would have undesirable effects the benefit of ceramic blades is obvious.

The initial higher cost of the ceramic blade is more than offset by its longevity.

The Vibroslice instrument is supplied with a blade holder suitable for both standard stainless steel blades and also our advanced ceramic blades. Sample blades of both types are included.

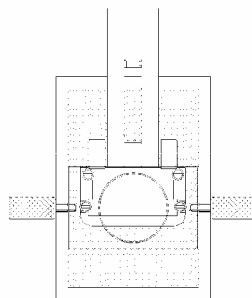


Standard blade holder (note: blade visor not shown)

When fitting blades care must be taken, as the blades supplied by Campden Instruments are very sharp.

The blade should be fitted by loosening the two screws on the arms of the holder and sliding the blade under each washer. The screws should not be over-tightened. See illustration above

The blade holder may be fitted with a transparent plastic 'Clearview' visor. This performs two functions. It will reduce the possibility of accidental cuts to the operator. The visor also performs a secondary function when the head is vibrating; if the visor is in contact with the bath solution, it will maintain a ripple-free surface allowing distortion-free viewing of the sectioning process. This is a particular advantage when the microscope attachment is being used. When slicing the blade should be positioned at the correct height for the desired slice and the level of a.c.s.f in the tissue bath adjusted so that it 'sticks' to the underside of the visor by capillary action maintaining a clear and undistorted view of the specimen and vibrating blade during the slicing operation. This is illustrated below. The part number for the Clearview visor is listed in the Spare Parts and Accessories section at the end of this manual.



Use of the 'Clearview' visor

Please note that illustration above also shows an adjustable blade holder. The standard blade holder presents the blade at an angle that has been optimised over many years of experience, some researchers may, however, wish to set the blade at some other angle. Campden Instruments can supply an adjustable blade holder. With this attachment the user can set the blade at any angle between horizontal and vertical. A protractor is also supplied to allow the angle to be gauged. Due to the extra mass of the adjustable blade holder and nosepiece configuration a slight reduction in cutting performance may be experienced when they are

used. This can generally be overcome by using a lower oscillation frequency. Part numbers for this option are listed in the Spare Parts and Accessories section at the end of this manual.

Operation

Before use, check that the voltage setting at the rear of the instrument is correct for your voltage supply.

1. Vibroslice with Hand Advanced tissue bath HA752

Fix a new blade in the blade assembly and fix the specimen holder in the bath. Care should be taken when setting up the instrument and when replacing the blade. You may wish to pre-set the height of the first cut by lowering the blade until it just touches the stage and then raising it the required distance.

Remove the brain or other tissue and trim as necessary.

Glue the tissue block to the specimen holder using a thin film of cyanoacrylate (Super glue) adhesive.

Pour cold, freshly oxygenated physiological saline into the bath until the tissue block is immersed.

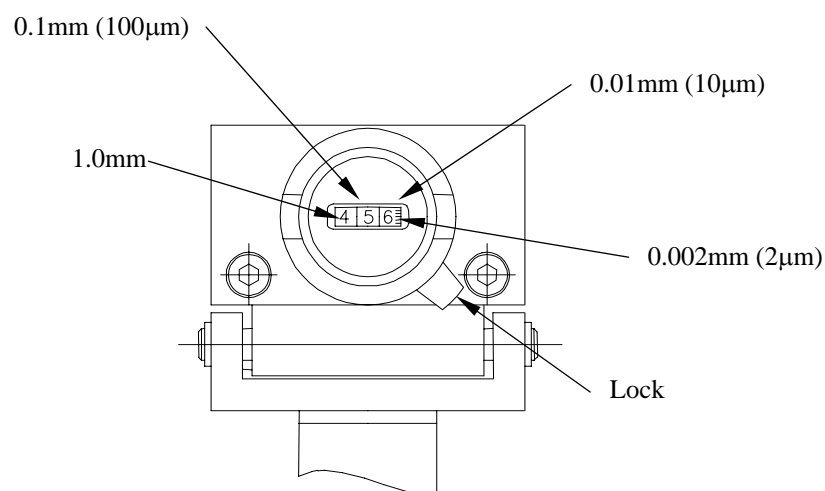
Set the blade vibrating by depressing the non-latching foot switch and adjust the vibration frequency as required.

Advance the tissue block onto the blade using the handle. When the blade begins to cut you may find it necessary to adjust the blade frequency to a more suitable rate.

When the cut is finished, the tissue block should be retracted from the blade by reversing the rotation of handle. You may wish to remove the tissue slice before retracting the tissue block.

Lower the blade by the required slice thickness. The numerals on the height adjustment knob represent 10 μ m, the small sub-divisions being 2 μ m as shown below.

Please note that the increments specified are nominal values only.



Repeat the above steps until you have sufficient slices, or you have run out of tissue.

2. Vibroslice with Motorised Advance tissue bath MA752

Operation of the MA752 Vibroslice is generally the same as the HA752 except that an electric motor removes the chore of repeatedly advancing and retracting the tissue bath by hand. The bath feed motor is controlled by a three-position switch which controls bath feed direction and a knob (graduated 1-11) that varies the speed of the bath advance.

Push the switch upwards for bath 'advance' to cut the tissue. When the cut is finished set the switch to the centre 'off' position. Reverse the bath by moving the switch down to 'bath reverse', and holding it down as this position is biased to 'off'. Limit switches will stop the carriage automatically at the limits of travel.

The speed of the bath advance is continuously variable. The speed of the bath reverse is fixed.

3. Preparing Tissue

The interval between death and the immersion of the glued tissue should be kept as short as possible to minimise anoxic damage, however total failures are more often due to clumsy handling than to anoxia.

Trimming the block is important because the orientation of the slices is determined by that of the surface glued to the stage. Parasagittal cuts have been found useful for transverse slices of rat dorsal hippocampus (glue down the cut midline) and for preserving the planar dendritic trees of Purkinje cells in folia of the cerebellum close to the midline (a Parasagittal initial cut should be made which removes the lateral lobes). In some cases it is easier to dissect the target structure free before mounting it on the stage. In others leaving the adjacent tissues on can give useful mechanical support (e.g. with transverse hippocampal slices); Tough connective tissue can cause trouble and should be cut off, or the block can be oriented to keep it out of the way of the cutting of the target structure. In a few special cases, additional support may be needed from external agents, such as embedding in Agar, or in formalised albumin (fixed tissues only).

3.1 Fixing Tissue

The block of tissue must be fixed firmly to the stage. Apply a thin film of cyanoacrylate (Super Glue) adhesive to the stage over an area large enough to accommodate the whole of the cut surface of the tissue block.

Two common problems associated with fixing tissue are:

(i) The tissue block floats away during cutting. This means that the glue has not bonded properly. This is usually due to the specimen being too wet. Use filter paper to draw off the excess liquid from the block before placing it on the stage.

(ii) The glue forms a rigid film up the side of the block, interfering with the cutting process. This probably means that too much glue has been used.

4. Preparing the Tissue Bath

Slices are cut under liquid to lubricate the blade as it cuts, and in the case of fresh tissue, to avoid anoxia. The tissue block should be covered to a depth of approximately one millimetre; much more than this reduces visibility due to rippling of the liquid surface.

The Vibroslice is supplied with a perfusion tube; this should be connected to a suitable gas supply and hooked over the side of the bath (away from the vibrating blade) so that the a.c.s.f. solution can be continuously perfused.

Cutting fresh tissue under cold (<8 degrees centigrade) physiological saline improves the quality of recordings, presumably by reducing the metabolic rate and avoiding anoxic damage. Keeping the saline on ice while oxygenating it may be enough. However, the saline warms up remarkably during the cutting process (to 10 degrees centigrade in 5-15 min depending on conditions). An ice-cold stainless steel block in the bottom of the chamber can help, as can freezing the whole cutting chamber with a few mm of physiological saline in the bottom (some spare chambers are useful in this case). Chilling can cause condensation on the stage interfering with gluing the tissue block; either wipe it dry at the last moment, or keep it out of the freezer and insert it in the chamber just before slicing. In the latter case a silicone rubber insert can be used to leave a gap in the frozen saline. A simpler solution is to buy the 764 Peltier cooling unit which has been designed to fit onto the Vibroslice.

5. Speed adjustment

Three factors govern the cutting action: The frequency of the blade vibration (oscillation), the speed of advance of the tissue block onto the blade and the amplitude of the blade movement. The vibration frequency rarely needs changing. The fastest setting is generally suitable for slices of fresh brain over 200µm thick. Thinner slices may benefit from slower vibration.

The rate of cutting, which is determined by the speed of the tissue advance, is one of the most important factors in successful slice cutting. It should be relatively slow (1 turn every few seconds). If it is too fast the blade tends to deform the tissue, often pushing it over rather than cutting it cleanly. What is meant by 'too fast' depends on the local consistency of the tissue; it tends to be slower in tougher tissues. Sometimes local areas of tougher tissues may require withdrawing the blade a little before resuming the cut. It always pays to monitor the tissue block as the cut progresses to check that all is well; a magnifier or dissecting microscope may help, as will good illumination.

The blade movement amplitude is fixed by an eccentric cam to 1mm, this has proved suitable for most applications.

If the blade consistently deforms the tissue block rather than cuts it, several possibilities should be considered. The simplest is to use a shorter block of tissue so that the cuts are made closer to the rigid support of the stage. In extreme cases extra support may be needed, most simply by leaving adjacent tissues attached. In a few cases the tissue may need to be embedded in Agar, or perhaps fix a suitable support on the stage next to the tissue, such as cork or pith (not expanded polystyrene as this disintegrates in contact with cyanoacrylate adhesive). If tissue block deformation is a regular problem, it may be worth modifying the stage or blade holder, in which case contact Campden Instruments for advice.

6. Slice Thickness

Slice thickness depends on why they are being cut. For most physiological studies on brain slices, the thickness is a compromise between anatomical integrity (better in thicker slices),

and oxygenation of the tissue (better in thinner slices). There are several experimental and theoretical accounts of the 'limiting thickness' of tissue slices, which is the thickest slice with a non-anoxic centre (eg; Warburg, 1930; Elliott, 1969; Harvey, Schofield & Brown, 1974). Most researchers usually use 400µm brain slices equilibrated with 95% O₂, 5% CO₂ at 35 degrees centigrade. If thicker slices are essential, then consider reducing the temperature to reduce the metabolic rate, or use a smaller animal. Slices of up to 750µm have been described (Halliwell, 1975). Thinner slices of around 100µm may be needed for applications involving direct visualisation of cells (Yamamoto & Chuko, 1978; Keenen et al, 1988; Konnerth, 1990), or where the problem is to improve the penetration of reagents for horseradish peroxidase labelling.

7. Removing Slices

Slices can be transferred as they are cut, or accumulated on the stage until the end of the run. A small stainless steel or plastic spatula ground to a fine edge resembling a chisel is useful to help slide the slice on and off. Alternatives are fine paintbrushes to wrap the slices on or wide-mouthed Pasteur pipettes to suck the slice up in a column of saline.

References

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2. Halliwell, J.V. (1975) J.Physiol. 246, 91-93P.
3. Harvey, J.A., Schofield, C.N. & Brown, D.A. (1974) Brain Res. 76, 235-245.
4. Konnerth, A. (1990) Trends Neurosci. 13:321-323 (1990).
5. Keenan, C.L., Chapman, P.F., Chang, V.C. & Brown, T.H. (1988) Brain Res. Bull. 21,373-383.
6. Warburg, O. (1930) pp75-93 in The Metabolism of Tumours. New York.F
7. Yamamoto, C.A. & Chujo, T. (1978) Exp. Brain Res. 31, 299-301.

Maintenance

The robust construction of the Vibroslice means that little maintenance is necessary apart from keeping it clean. The stage should be scraped clean after each use, removing all traces of glue. All the wetted parts, especially the blade holder, should be rinsed in distilled water and dried.

Do not use any solvents such as acetone or chloroform.

After a long period of use, the drive belts may deteriorate and break. To check or change the belts, disconnect the instrument from the electrical supply and remove the screws securing the top cover. Remove the top cover and inspect the condition of the exposed belts - change as required. Note that the HA752 manual version has two belts to drive the blade head and the MA752 motorised version has two additional belts to drive the bath slide. Replace the top cover and securing screws.

Specifications

Section Thickness:	Minimum 20 μ m (fixed tissue), 50 μ m (fresh tissue) Maximum 700 μ m	
Step size:	10 μ m	
Chamber dimensions:	55mm x 82mm x 31mm	
Chamber volume:	140ml.	
Bath advance speed (MA752):	0.14 to 2 mm/sec continuously variable	
Bath reverse speed:	2 mm/sec	
Vibration speed:	60-4500RPM	
Vibration displacement:	1mm	
Power requirements:	220V/240V, 50Hz, 0.5A (Selectable via switch at rear) 110V/130V, 60Hz, 0.5A	
Permissible Voltage Tolerance:	+10%, -6%	
Current Consumption:	HA752:	0.07A (Average)
	MA752:	0.09A (Average)
Power Ratings:	HA752:	15W
	MA752:	20W
Fuse Ratings:	HA752:	Mains 220V/240V ac, 250mA, type 'T' 20mm Mains 110V/130V ac, 250mA, type 'T' 20mm
	MA752:	Mains 220V/240V ac, 500mA, type 'T' 20mm Mains 110V/130V ac, 500mA, type 'T' 20mm

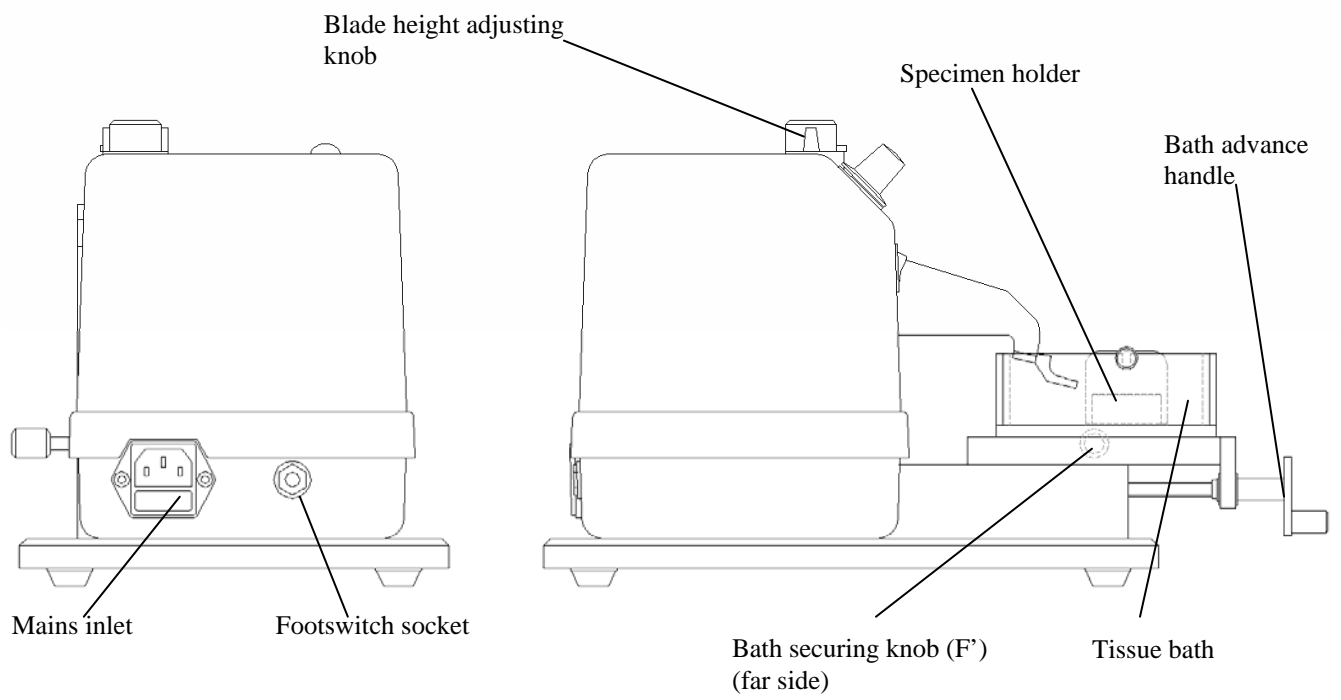
Each Vibroslice is supplied with the following:

- Tissue Bath
- Tissue Holder
- Perfusion tube
- 10 Stainless steel Blades
- 2 Ceramic blades
- Foot Switch
- Mains Lead
- Hexagonal wrench (2 off)
- Instruction Manual

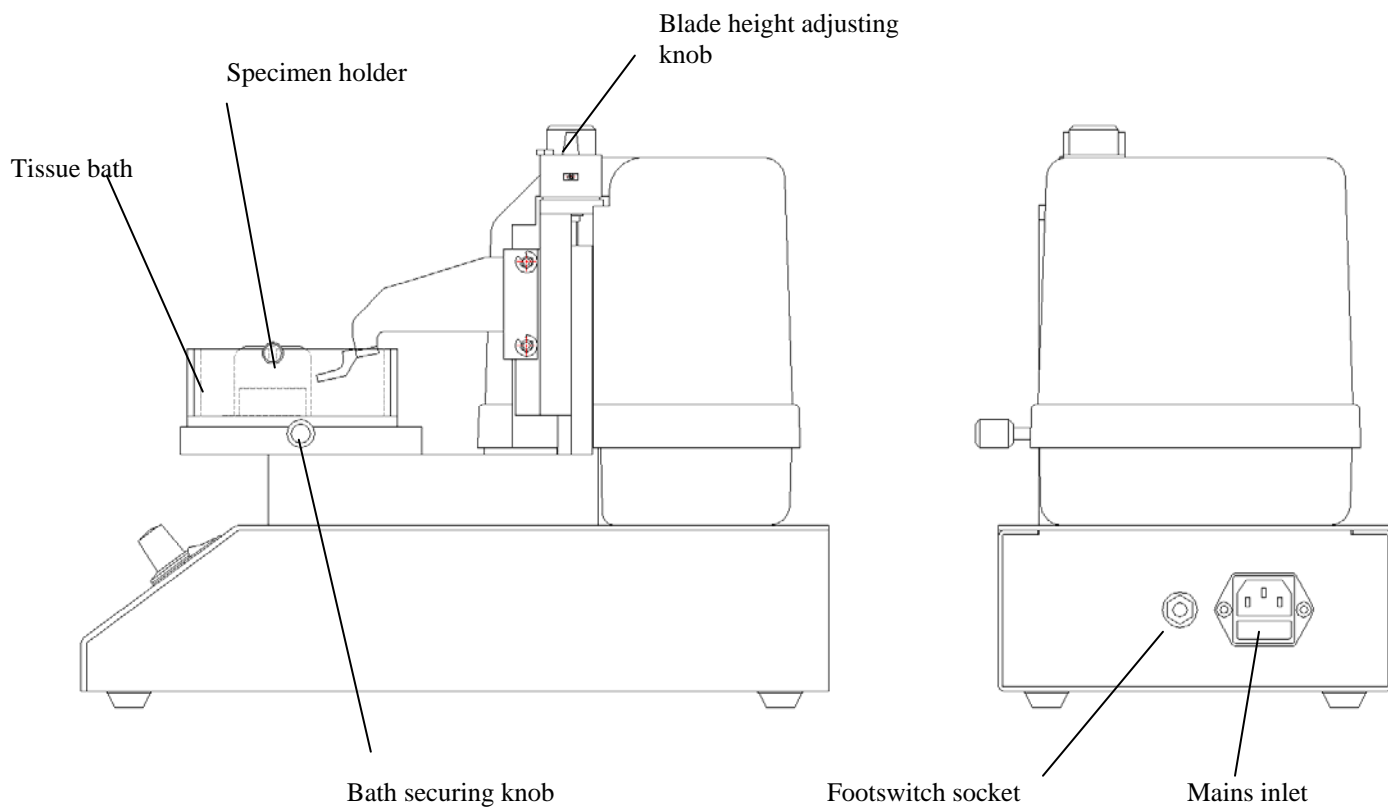
Spare Parts and Accessories

When ordering, please order by part number and description

Manual Vibroslice	HA752
Motorised Vibroslice	MA752
Peltier Cooling Bath for Vibroslice (low power)	765LP
Peltier Cooling Bath for Vibroslice (high power)	765HP
Stainless steel blades (pack of 50)	7550/1/SS
Ceramic blades (pack of 5)	7550/1/C
Nose piece (standard)	752/7
Nose piece (adjustable)	752/7A
Blade holder (standard)	7550/9
Blade holder (adjustable)	7550/9A
Specimen Holder	752/2A
Adjustable specimen holder	752/2A/A
Tissue Bath	752/2B
Autoclavable tissue bath (121° Celsius)	752/2AC
Bath mirror	752/2BM
Bath perfusion tube	752/2BPT
Tissue Holder and Bath (standard) assembly	752/2AB
Drive belts for MA752 (pack of 8, two sets of 4)	752/4
Drive belts for HA752 (pack of 4)	752/5
Blade visor (standard) (pack of 5)	7550/6
Blade visor (adjustable blade) (pack of 5)	7550/6A
Protractor for setting blade angle (pack of 5)	752/069



HA752 Vibroslice



MA752 Vibroslice

