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KnifeMaker II Operating Instructions including Spare Parts Catalogue

REICHERT-JUNG OPTISCHE WERKE AG AUSTRIA

A Cambridge Instruments Company



KnifeMaker II Operating Instructions including Spare Parts Catalogue

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Spare Parts Catalogue



PLEASE READ THIS MANUAL CAREFULLY BEFORE ATTEMPTING TO USE THE INSTRUMENT.

This instruction manual describes the function and operation of the 2178 Knife Maker II. All users, even experienced users of the 7800 Knife Maker, are recommended to read this manual.

1. IMPORTANT USER INFORMATION

If you have any comments on this manual, we will be pleased to receive them at:

REICHERT-JUNG Optische Werke AG Hernalser Hauptstrasse 219 A-1171 Vienna, Austria

Since product development and improvement are continuous, REICHERT-JUNG reserves the right to make changes in the specification without notice.

WARRANTY AND LIABILITY

REICHERT-JUNG guarantee that the product delivered has been thoroughly tested to ensure that it meets its published specification. The warranty included in the conditions of delivery is valid only if the product has been used according to the instructions supplied by REICHERT-JUNG. We can accept no liability for loss or damage, however caused, arising from the faulty or incorrect use of its products.

SAFETY WARNING

For the safety of all laboratory staff, the user is advised to keep the Knife Maker II clean and free of any small pieces of glass. Always dispose of discarded glass knifes safely.

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Using the LKB KnifeMaker II, strips of 25mm wide, high quality glass of up to 10mm in thickness are scored transversely then broken into squares or rhombus-shapes. Each square or rhombus is returned to the KnifeMaker II and scored diagonally, then fractured into two knives each with a straight, sharp edge for use in ultramicrotomy or histology.

The LKB KnifeMaker II utilises the wellproven design principles of the original LKB KnifeMaker. This popular instrument, which made manual knife-making techniques obsolete, standardised the production of glass knives by defining the position of the score on the glass and by applying the optimal pressure at the correct locations to ensure a slow controlled break. These principles have been further refined to produce the balanced break method of breaking the strips, to incorporate new developments in scoring and to simplify the entire operation of routine knife-making.

With the balanced break method, KnifeMaker II routinely produces straight-sided squares of unprecedented reproducibility. It also allows greater flexibility in the choice of score before the final fracture into two knives. A choice of three preset score lengths are provided on KnifeMaker II; a short symmetrical score producing a long free break, the traditional asymmetrical medium-length of score, and a long score giving a short, but well-controlled free break. When gentle pressure is applied directly under the score, the square fractures to form two usable knives.

Although the position of each of these three scores is fixed, the position of the glass square can be adjusted to allow for the specific requirements of the user. By controlling the position of the glass square relative to the score, knifemaking can be optimised for ultrathin, semithin or frozen sectioning. For security in a multi-user environment, these adjustments can be locked to ensure the reproducible production of high quality knives.

The traditional knife angle for ultramicrotomy is 45°, however it is often recommended that a larger angle should be used for specimens which prove difficult to section. KnifeMaker II retains the versatility of the original LKB KnifeMaker in producing a range of knife angles. By setting the click-position glass holder, a rhombus can be made from the strip to provide knife angles larger than 45°.



2.INTRODUCTION TO KNIFEMAKER II



3.1 Unpacking KnifeMaker II

1. Ensure that the carton is the correct way up, then unpack the contents. Handle the box of glass strips with care.

 Remove the packing materials but do not discard until each item has been checked against the Packing List supplied with the instrument. Retain the packing if further transport can be forseen. There are two knives taped to the side of the instrument. Leave these in place; they are described later in 3.3, Preliminary Checks.

3. Check each item for any sign of transit damage, and immediately report any such damage to your local LKB representative and the transport company concerned.

4. Open the box of glass carefully. For the protection of all users, it is strongly recommended that all sharp-edged metal staples should be removed from the carton with pliers.

3.2 Installation

 Place the KnifeMaker II on a sturdy laboratory bench or table where it will not be affected by movement from other laboratory equipment, such as a centrifuge. During knifemaking, strong vibrations transmitted to the KnifeMaker II from the surface of the bench could result in imperfect knife edges.

 KnifeMaker II is completely assembled on delivery. Unpack the accessories, and retain the spare parts and tool kit safely until they are required.

3.3 Preliminary checks

3. INSTALLATION OF KNIFEMAKER II

> KnifeMaker II is adjusted in the factory before packing. The two knives taped to the side of the instrument have been produced on your KnifeMaker II, using the long symmetrical score to break the square into two knives. These knives demonstrate that the performance of the instrument was checked and found satisfactory at the factory. However, it should be noted that if severe handling has occured during transit, it is possible that these adjustments may have been disturbed. When using KnifeMaker II for the first time, the following procedure should be used.

2. Read through chapters 4 and 5 to learn about knifemaking with KnifeMaker II.

3. Follow the instructions for routine knifemaking in chapter 6, sections 1, 2 and 3 and make some squares using the balanced break method.

4. Continue to follow the instructions in chapter 6, section 4, and make a 45° angled knife.

5. If the knife edges are not straight or are unsatisfactory for any other reason, refer to chapter 7, and after reading the introduction, make a test score (chapter 7, section 2).

6. Once the test score is satisfactory, follow the instructions in chapter 7, section 3 for adjusting the width of the counterpiece.

7. If KnifeMaker II is to be used only for Lnives for cryoultramicrotomy, refer to chapter 8.

8. When all adjustments to KnifeMaker II are complete, the settings can be protected by replacing the thumb screws on the counterpiece adjustment controls with the locking screws and washers supplied. Use a screwdriver to tighten the screws.



4.1 The Balanced Break

In the balanced break method, a standard strip of glass, 400mm in length, is scored and broken into two equal halves, each 200mm in length. With an equal mass of glass on each side of the score, the break is balanced and the freshly fractured surfaces are plane. By continuing to divide each piece produced into two equal halves, up to 16 squares can be made (Fig.1). All squares produced have straight sides and precise right-angled corners. The balanced break method is used only for making squares. Rhombus-shapes, which are used for angles other than 45', are sequentially broken off the end of a strip of glass (Fig.2). This break is not balanced, so the freshly fractured surfaces show some curvature and the corners are irregular.



Fig.2 The sequential break

4.2 Scoring and breaking principles

Producing good glass knives routinely depends on a supply of reproducible squares or rhombi, an accurately positioned score and controlled pressure precisely applied to make the break. KnifeMaker II gives the user the choice of three scores, each one factory-set for making knives from squares and rhombi. Each score is preset in a position equidistant from the two support studis in the scoring head which rest on the glass and hold it in a horizontal position (Fig.3). To initiate the fracture and break the square, gentle pressure is applied directly under the score by raising the two breaking pins (Fig.4).



Fig.3 Score equidistant from support studs





While the location of the score is preset, the square or rhombus can be moved relative to the score to allow for individual requirements from the knife. For routine knifemaking, the score is directed slightly off the diagonal, so that the fracture forming the knife edge occurs on one of the sides of the square, a short distance from the corner (Fig.5). This leaves a narrow "counterpiece" opposite each knife. Ideally, the two counterpieces formed from a square should be equal in width, indicating that the fracture is symmetrical.



Fig.5 Formation of two knives

As a general rule, the knife edge is straighter when the fracture occurs close to the corner, and the counterpiece is small. Because squares formed by the balanced break method have straight sides, it is possible to keep the counterpiece below 0.4mm as a routine. When making a knife for cryoultramicrotomy, the counterpiece can be reduced still further, or the break can be made exactly into the 90° corner.

4.3 The real knife angle

For scoring the square, all three scores stop some distance from the corner. When pressure is applied under the score, the fracture is initiated and is seen at first as a deepening of the score. The fracture extends towards the corners of the square, following the line of the score. Where the score ends and the break is "free", the fracture deviates from the line of the score to curve away from the corner, towards one of the edges of the square. This results in the *real included angle* of the knife being somewhat greater than the *angle* of scoring (Fig.6). The real angle of the knife increases as the score is moved further from the diagonal, . that is, when the counterpiece is larger. For example, when preparing knives from a square, the real angle of the knife formed when the counterpiece is the recommended 0.4mm in width, is 45°, plus 8 to 12°. Increasing the width of the counterpiece results in an even larger real angle. Reducing the counterpiece below 0.4mm results in an angle closer to the scoring angle of 45°. The same principles apply to a large angle of knife prepared from a rhombus.



Fig.6 Real knife angle

4.4 Length of useful edge

When a glass knife edge is examined under darkfield illumination using a stereomicroscope, it can be seen that the central part is most useful for ultrathin sectioning (Fig.7). The right side of the edge has visible stress marks which reduce the quality of the knife, and the left corner is also unsuitable for sectioning because of the stress line. The length of useful edge is found to be longer in a larger angle of knife made from a rhombus, and in all knives produced with small counterpieces. The useful length of edge is also enhanced by using the damping pad to absorb the shock of the fracture.







5. KNOW YOUR KNIFEMAKER II

All scoring and breaking mechanisms for knifemaking are assembled on a heavy vibration-absorbing base casting (Fig.8).

For producing squares or rhombi of different angles, the glass strip is securely held in the strip holding plate. This holder, which is manufactured from a plastic material to prevent damage to the strip edge, has a locating support to hold the strip in the correct position (Fig.9). To allow different knife angles to be made, the strip holder can be set at any one of five preset click positions which present the strip to the scoring and breaking mechanism at different angles. The position selected is locked by tightening the locking nut.



Fig.9 Strip holding plate

To make squares with plane surfaces from strips of glass, the balanced-break method is used. To position each length of strip for dividing into two equal halves, there are four retractable arresting studs in the stainless steel cover plate. The strip is inserted into the strip holding plate with the end positioned against the appropriate stud. The glass is then scored and broken into two equal halves, each with a straight freshly-cleaved surface. For sequential breaking of rhombi from strips, the end of the strip is positioned against the nearest retractable arresting stud. The scoring head is lowered down on to the glass by turning the clamping handle. Other than gravity, no force is applied during its downward travel. When the handle is in the rear position, the scoring head is raised (Fig.10). Moving the handle to the vertical position, lowers the scoring head. Not not the glass, which is levelled by contact with the two support studs under the scoring head. When the handle is brought forward to the horizontal position, the scoring head is pulled and locked against the clamping head to firmly clamp the glass in position. Excessive pressure to clamp the glass is unnecessary.



Fig.10 Scoring head movements

The mechanism for scoring the glass is held within the scoring head. A tungsten carbide wheel, mounted within a cartridge on the scoring shaft, is located in a central position between the two support studs (Fig.11). When the scoring shaft is pulled out, the wheel makes a score on the glass. The pressure of the wheel on the glass can be adjusted to give an optimal depth of score. The scoring shaft runs on a cam which controls the length of score. On KnifeMaker II, there are four different cam profiles. Each score length is indicated by a symbol, and is selected by turning the cam selector knob until the appropriate symbol is uppermost.



prepare knives of any angle from squares or rhombi, and is the preferred score for making any angle of knife larger than 45°.



This symbol indicates the short length of score. This score will be found most useful when making a 45° knife for cryoultramicrotomy from a square prepared by the balanced break of the strip. The score can be centrally placed on the square and directed along the line of the diagonal. Knives can be formed very close to the corner, leaving an extremely narrow counterpiece, or the break can be made exactly into the corner.

This symbol indicates the long length of score. This is the preferred score for the routine preparation of a 45' knife from a square, for ultramicrotomy and histology. It is also suitable for making 45' knives for cryoultramicrotomy. This score gives a very short free break to each corner of the square, and enables corner-tocorner breaking. It should also be used for breaking angles smaller than 45'.

After scoring the glass, the break is initiated by applying gentle pressure directly under the score (Fig.11). Two breaking pins are located under the score in a central position. By moving the breaking handle upwards, these pins are raised slightly to apply pressure under the score, just sufficient to break the glass. The breaking handle should be turned slowly and evenly until the glass fractures.

Fig.11 Scoring head



This symbol indicates the score used when preparing squares or rhombi from a strip of glass.



This symbol indicates the traditional asymmetrical score. This medium length of score can be used in routine knifemaking for ultramicrotomy and histology. It can be used to To make the final break into two knives, the square or thombus is inserted between two graduated guide forks which form part of the counterpiece adjustment controls (Fig.12). The back control is spring-loaded and can be moved towards the operator to hold the glass firmly, or away to release the glass. The front control cannot be moved in this way. The graduated guide fork on the back control must be set to the correct spring tension in order to hold the glass firmly in position.





Fig.12 Spring-loaded guide fork

The front graduated fork can be set by loosening the locking screw (Fig.13). Pulling the look towards the operator, moves the square or rhombus to increase the distance between the end of the score and the corner of the glass, thereby increasing the length of free break. Conversely, pushing the plate away from the operator decreases the length of free break. When the front graduated plate is adjusted, a similar adjustment must be made on the back plate to compensate.

The score is in a fixed position; however by using the two counterpiece adjustment controls (C₂ adjustments), the corners of the square or thombus can be moved sideways to achieve the correct scoring and breaking positions. When the thumb screw is loosened, the counterpiece adjustment control can be turned to move the glass corner laterally (Fig.14). An arrow on the control indicates the direction of movement for







Fig.14 Adjustment of counterpiece width

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Located on the scale plate of the front counterpiece adjustment device is the rubber damping pad. Operated by a lever, this pad is moved forward to contact and support the corner of the glass after scoring but before breaking. During the break, this pad absorbs the shock and ensures an increased length of useful cutting edge on the knife. To achieve the maximum damping effect from the pad, it is important to position it correctly against the corner. The pad should be pressed against the corner until a clear indentation in the pad is visible (Fig.15). The damping pad can be used on all knife angles except where the counterpiece is less than 0.3mm in width, since in this situation, the pad will contact the edge of the knife and will damäge it.



Fig.15 Damping pad

6.1 Hints on handling glass strips

For the best results from your KnifeMaker II, it is recommended that glass strips supplied by LKB Produkter AB are always used. Strips for ultramicrotomy are produced from selected glass made by a special manufacturing process. The thickness and quality of all glass used for strips is precisely controlled. The production of the strips follows strict tolerances ensured by careful quality control. Knifemaking is only reproducible when using glass strips with tolerances strictly controlled.

All strips supplied by LKB are 400mm long and 25mm wide. They are available in thicknesses of 6.4 and 8mm for ultramicrotomy, and 8 and 10mm for histology. Strips supplied for ultrathin sectioning are produced to a higher specification than those for semithin sectioning, therefore histology quality strips should *not* be used for ultramicrotomy. However, the higher quality strips for ultramicrotomy can be used for histology. Part numbers for re-ordering glass strips are given in Appendix 2.

LKB glass strips are individually wrapped in tissue paper, and packed in a strong carton, closed with metal staples. For the users safety, these staples should be completely removed from the carton before taking the strips from the box.

When handling the strips, avoid contacting the edges if these will finally form the knife edge. The strips and squares can be held by the wide upper and lower surfaces (Fig.16). Never touch the corners of a partly broken strip, square or rhombus, since these will form the knife edge.

To protect the edges, each strip is wrapped separately in tissue paper. Although the strips are washed before packing, often an opaque film is visible on the strips when they are umpacked. This film, which is the result of contact of the paper on the glass, should be removed by careful washing before the strips are used for knifemaking.



Fig.16 Handling the glass

6. ROUTINE KNIFEMAKING

Most laboratories have their own techniques for washing the glass. The method described here is recommended for general use. Wash each strip separately in cool tap water which has a low concentration of mild laboratory detergent added to it. Use a soft brush or cloth, and take care not to touch the sharp edges of the strip. Completely rinse off all the detergent, then flush thoroughly with distilled water. Dry gently with a clean soft lint-free glass cloth, which will not scratch the edges of the strip.

6.2 The alignment of the glass in KnifeMaker II

In the following operating instructions, the user is recommended to always place the glass strip and the square or rhombus into the KnifeMaker II in the same specific orientation.

When making 45° angled knives from a square produced by balanced breaking, you can choose to use either the freshly fractured surface or the original edge of the strip to form the knife. Therefore, if you are using conventional methods of Knifemaking with the score directed slightly to the right of the corner, the square should be turned until the side chosen to form the knife is to the right (Fig. 17). Once you have decided which side to use, it is advisable to standardise and use the same orientation always. Whether you choose the freshly fractured surface or the original edge of the strip, the unscored side should be upwards since this forms the left (good) side of the knife edge. When breaking the strip into squares, always place the strip into the strip holder plate with the scored edge down (Fig. 18).





For knives other than 45°, a rhombus is prepared by sequential breaking from the strip. Since this method gives a freshly fractured surface which is non-plane, it is essential that the edge of the original strip is used to form the knife (Fig. 19). As with 45° knives, the left side of the knife edge should be formed by the unscored side of the strip. To allow for this orientation when making angles *larger* than 45°, the strip must be placed into the strip holding plate with the scored edge *upwards* (Fig. 20).



Fig.18 Alignment of the strip









Conversely, for angles *smaller* than 45° , the scored edge of the strip must be *downwards* when placed into the strip holding plate (Fig.21).



Fig.21 Strip aligned for small angles

By standardising the alignment of the glass strip, square and rhombus, all knife edges will be the same reproducible high quality, and any adjustments required to the KnifeMaker II will be easier.

6.3 Making squares using the balanced-break method

This method of breaking 400mm strips of glass produces squares with a freshly fractured surface which is close to a true plane. It is recommended that this method is always used when making 45° angled knives of 6.4mm and 8mm width for ultramicrotomy and cryoultramicrotomy, and when making 45° knives up to 10mm in thickness for semi-thin sectioning in histology.

1. The glass strips should be washed and dried as described in 6.1.

 If necessary, losen the locking screw and adjust the strip holding plate to make 45° angled knives. Check that the scoring shaft is pushed in, and the breaking handle is in the anticlockwise stop position.

 With the scored edge of the strip downwards, fit the strip into the strip holder by pushing it against the locating support (Fig.22). Position it so that the left end of the strip is in contact with the first (far left) arresting stud.



Fig.22 Strip aligned for first fracture

4. Use the clamping handle to lower the scoring head onto the strip and to clamp the glass in position (Fig.23). To ensure that the glass strip is levelled by the scoring head and therefore clamped correctly, do not touch the glass during this operation.



Fig.23 Lowering the scoring head

5. Select the correct length of score for scoring the strip (Fig. 24).



Fig.24 Score indicator

6. Pull out the scoring shaft to make a single score on the glass strip. Do not push in the shaft again since this will make a second score, and the resultant break will be uneven.

7. Turn the breaking handle upwards in a clockwise direction slowly and evenly (Fig.25) until the glass breaks, then return the breaking handle to the stop position.



8. Place the right hand under the scoring shaft to prevent the scoring head dropping on to the glass and, with the left hand, use the clamping handle to release and raise the scoring head. Push in the scoring shaft ready for the next score.

9. Remove one of the 200mm lengths of glass, and position the other strip in the strip holder so that the left end is in contact with the second arresting stud (Fig.26). NOTE: If the length of the original strip was not 400mm, only the left half of the strip will be exactly 200mm long.

10. Lower the scoring head onto the glass and lock in position, remembering not to touch the glass strip. Score the strip as before, then slowly break into two 100mm lengths. Raise the scoring head and remove one of the 100mm pieces.

11. Position the other 100mm strip in the strip holding plate, with the left end in contact with the third arresting stud. Clamp the strip in position, then score and break as before. Remove one of the 50mm strips.

12. Position the left end of the other 50mm strip against the fourth arresting stud. With the right hand, hold the strip *lightly* against the edge of the strip holding plate. Lower the scoring head onto the glass and lock. To ensure



Fig.26 Strip aligned for 2nd and 3rd fracture

that the strip is correctly levelled by the scoring head, avoid any excessive downwards pressure on the strip while clamping.

13. Place the retrieving fork under the end of the strip, then score and break the strip as before. Place the right hand under the scoring shaft to prevent the scoring head dropping on to the glass, then raise the clamp head. Push in the scoring shaft.

14. Use the retrieving fork to remove the left square (Fig.27). This final break produces two 25mm squares, ready for breaking into two 45° knives.

15. Continue to make 25mm squares from the remaining strips of glass, as necessary. Up to 16 squares can be made from a 400mm long strip, however if the two squares formed from the ends of the original 400mm strip have uneven edges, they should be discarded.

16. Handle the squares with care. Do not touch the corners or edges which will later form the knives. Avoid all accidental impacts. To avoid touching the edges of the square, the knife retriever can be used to move the squares.



Fig.27 Using the retrieving fork

6.4 Making knives from squares

1. Push the back spring-loaded glass holder to the disengaged position (Fig.28). Check that the damping pad is disengaged.



Fig.28 Disengaging the guide fork

 If you are using KnifeMaker II for the first time, set the front graduated fork to 8 on the scale if you have chosen to use the long score, or to 10 for the traditional medium score.

 Align a 25mm square so that the edge chosen to form the knife is to the right with the scored edge downwards (Fig.29). Place it on top of the breaking pins and insert it into the front guide plate.



Fig.29 Alignment of the square

4. Use the left hand to hold the square in the front guide plate. Use the right hand to move the spring-loaded back guide plate gently towards the operator to hold the square securely. NOTE: When KnifeMaker II is used for the first time, the back guide plate should be adjusted so that the spring tension is sufficient to hold the glass securely. To do this, fit a square, then move the back guide plate into the engaged position. Loosen the locking screw and push the graduated plate to touch the glass. Gently push the guide plate holder back into the disengaged position, then slide the scale plate 2mm towards the operator. Tighten the locking screw. This setting can remain for all knives of this angle, however if an adjustment is made to the front graduated plate, the same adjustment must be made to the back plate.

5. Check that the retrieving fork is in place under the square.

6. Use the clamping handle to lower the scoring head on to the square, and lock.



Fig.30 Long symmetrical score

 Select the required length of score. You can choose either the medium length traditional score, the short score or the long score, however for routine knifemaking, the long symmetrical score is recommended (Fig.30).

 Pull out the scoring shaft to score the glass. Do not push the shaft back in since this will make a second score and the break will be uneven.

9. Turn the damping pad lever until the corner of the square can be seen to make a small indentation in the pad (Fig.31). Always set the damping pad *after* scoring.

10. Move the breaking handle upwards very slowly and evenly to break the square, then return the breaking handle.

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Fig.31 Using the damping pad

11. Turn the damping pad lever to completely . remove the pad from the corner of the glass.

12. Place the right hand under the scoring shaft to prevent the scoring head from dropping on to the knives, and with the left hand, use the clamping handle to release and raise the scoring head. Push in the scoring shaft.

13. With the left hand, raise the knife retriever slightly to support the two knives, then with the right hand push the back glass holder to disengage it. Carefully remove the two knives on the knife retriever (Fig.32).



Fig.32 Using the retrieving fork

6.5 Making a rhombus from the strip

This method describes breaking strips into rhombus shapes, which will be broken subsequently into knives with an angle larger than 45. Knives of this larger angle are often more durable for cutting semithin or ultrathin sections. Also, a larger angle of knife has a longer useful cutting edge than a 45° angled knife. Glass strips up to 10mm in thickness can be broken in this way.

The method described is the sequential method of breaking strips, that is after halving the strip, rhombi of the required angles are fractured sequentially from one end of the strip. Rhombi cannot be made by balanced breaking.

 Push the back glass holder to the disengaged position. For breaking the strip for the widest angle of rhombus, it may also be necessary to lossen the locking screw and push back the graduated guide plate (Fig. 33).





 Loosen the knob locking the strip holding plate, and move the plate through the clickstops until it is correctly positioned for the angle of knife required. Tighten the locking knob.

 With the scored edge of the strip upwards (Fig.34), fit the freshly washed strip into the strip holding plate. To half the strip, position it so that the left end is level with the first arresting stud on the left side of the instrument.

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Fig.34 Alignment of strip for large angle knives

4. Lower and clamp the scoring head. Remember not to touch the strip during clamping or the strip may not be correctly levelled.

5. Select the score length for scoring the strip (Fig.35).



Fig.35 Score indicator for scoring the strip

6. Pull out the scoring shaft to score the strip. Remember not to push the shaft in again since this will result in a second score and an uneven break.

7. Move the breaking handle upwards slowly and evenly until the glass breaks. Return the breaking handle when the glass is broken.

8. Place the right hand under the scoring shaft to prevent the scoring head dropping on to the glass, then unclamp and raise the scoring head. Push in the scoring shaft ready for the next score. Remove the left half of the strip. 9. Position the right half in the strip holder plate with the left end of the strip touching the fourth (nearest) arresting stud (Fig.36). Lower and clamp the scoring head.



Fig.36 Strip positioned for sequential breaking

10. Place the retrieving fork under the end of the strip. Check that the score length selected is still correct for scoring the strip. Score the strip and break with a slow and even movement on the breaking handle. When the glass is broken, return the breaking handle.

11. With the right hand supporting the scoring shaft, unclamp and raise the scoring head. Push in the scoring shaft. Use the retrieving fork to remove the rhombus.

12. Continue to make as many rhombi as required following steps 9, 10 and 11. Return the strip holding plate to the position for making squares.

6.6 Making large angle knives from a rhombus

 When handling a rhombus, hold by the upper and lower surfaces, and avoid touching the corners and edges which will form the final knife edge. The retrieving fork can be used to lift the rhombus.

2. Turn the rhombus upside down and rotate it until the wider angle is at the front. The freshly fractured edge should be on the left (Fig.37), and the edge of the original strip on the right, with the score downwards. Place the rhombus in this orientation on top of the breaking pins in the front guide fork. NOTE: If KnifeMaker II has previously been used for making 45° knives, the front graduated guide fork may need to be adjusted when changing to angles larger than 45°. Instructions are given in chapter 7, section 4.



original edge of strip

Fig.37 Alignment of the rhombus

3. Use the left hand to position the rhombus in the front guide fork. Use the right hand to move the spring-loaded back guide fork inwards to hold the rhombus securely. If an adjustment has been made to the front graduated fork, then a similar adjustment must be made to the back graduated fork. Also, if the back graduated fork has been moved when breaking the strip, reset it to give the correct tension.

Make sure that the retrieving fork is positioned under the rhombus.

5. Lower the scoring head on to the glass, and clamp.

6. Turn the score selector to the scoring length required, You can choose either the traditional asymmetrical score or the short score, but for routine knifemaking, it is preferable to use the traditional, medium length of score (Fig. 38). It is not possible to use the long score on a rhombus.



Fig.38 Score indicator for traditional score

7. Score the glass. Remember not to push the shaft in again since this will result in a second score and an uneven break.

8. Turn the damping pad lever until you can see that the corner of the rhombus makes a small indentation in the pad.

9. Turn the breaking handle clockwise with slow and even pressure until the glass breaks, then return the breaking handle.

10. Turn the damping pad lever to move the pad away from the corner of the rhombus.

11. Place the right hand under the scoring shaft to prevent the scoring head dropping on the knives, and use the left hand to unclamp and raise the scoring head.

12. Gently raise the retrieving fork to support the two knives, then push the back guide plate to release the knives. Carefully remove the two knives using the retrieving fork.

6.7 Making small angle knives from a rhombus

Knives with angles less than 45° can be made from thombi formed from strips of up to 10mm in thickness. A knife with an angle such as 40° has an extremely short length of useful edge which is not durable. Also, smaller angles are more difficult to make than larger angles, and careful adjustment of Knife Maker II will be necessary. For these reasons, a small angle is not recommended for normal routine use. However, it may be necessary to use a small angle for cutting certain specimen types. Alternatively, a cryoknife can also be used. This is a sharp and reproducible knife, but it is not durable for sectioning resin embedded specimens. Instructions for making cryoknives are given in chapter 8.

1. Refer to steps 1 and 2 in section 6.5, the instructions for breaking rhombi from a glass strip.

 Loosen the knob locking the glass strip holder plate, and move the plate through the click-stops until it is correctly positioned for the angle of knife required (Fig. 39). Tighten the locking knob.

 With the scored edge of the glass downwards (Fig.40), fit the freshly washed strip into the strip holding plate. To half the strip, position it so that the left end is level with the far left arresting stud.



Fig.39 Positioning strip holding plate



Fig.40 Alignment of strip for small angles

4. Continue to follow Steps 4 — 12 in section 6.5, for making rhombi.

5. To make the knife, turn the rhombus anticlockwise so that the narrow angle of the rhombus is at the front. The freshly-fractured edge should be at the left, and the edge of the original strip to the right, with the score downwards (Fig.41). Place the rhombus on top of the breaking plus in the front guide plate. NOTE: If the KnifeMaker has previously been used for making other angles of knife, the front guide plate may need to be adjusted. Instructions are given in Chapter 7.5.

6. Use the left hand to position the rhombus in the front guide plate and use the right hand to move the back glass holder inwards to hold the rhombus securely.

NOTE: If an adjustment has been made to the front guide plate, then a similar adjustment must be made to the back guide plate.

7. Refer to section 6.6 and follow the instructions given in steps 4 and 5, for making knives from a rhombus.



Fig.41 Alignment of rhombus

8. Select the scoring length required (Fig.42). It is preferable to use the long symmetrical score, but it is also possible to use the short score or the medium traditional score.



Fig.42 Score indicator

9. Refer to section 6.6 and follow the instructions from step 7 to step 12.

6.8 Storing your knives

It is recommended that knives should be stored safely in a knife box with a lid until they are required. There, the edges will be protected from damage and dust particles, which destroy the edge. The sharp edge will be destroyed if it contacts any object. Special care should be taken when fitting troughs to the knife.

Since the sharpness of the edge gradually deteriorates, it is generally recommended that knives should be used for sectioning within the same day of their preparation. Storage for longer is not recommended since fresh new knives are quick and easy to prepare. Knives left over from the previous day can be used for trumming.

7.1 Introduction

For ultramicrotomy and microtomy, producing a good glass knife from a square or rhombus requires an accurately positioned score and slow controlled pressure precisely applied to make the break. With KnifeMaker II, each score is a preset length and is in a central position between the two scoring head support studs, ensuring an equal mass of glass on each side of the score. Minimal pressure is evenly applied directly under the centre of the score to break the glass into two knives (Fig.43). In KnifeMaker II, these conditions are preset to ensure standardisation and reproducibility in knifemaking, however some flexibility exists in the adjustment of the position of the glass in relation to the score. The following instructions describe the adjustment of the KnifeMaker II to produce good knives for routine sectioning. Chapter 8 describes the adjustments required for making good knives for crossectioning.



Fig.43 Breaking pressure

7.2 Making a test score on a square

7.GETTING MORE FROM YOUR KNIFEMAKER II

> 1. Turn the score selector to the score used routinely in the laboratory. You can choose either the medium, asymmetrical score, or the short or the long symmetrical score, NOTE: The break is initiated by applying pressure under the score, and it extends in both directions across the square following the line of the score. From the end of the score, the break is "free". The short symmetrical score length gives a long free break, and the long symmetrical score gives a short free break. Although some knives resulting from a long free break will be better knives, the procedure is less reproducible because the break is less well controlled. Therefore, for routine knifemaking from squares, the user is recommended to use the long score length (Fig.44).



Fig.44 Score indicator

 If either the long or short scores are to be used, set the front graduated fork at 3. If the traditional medium length of score is to be used, set the front graduated fork at 10. These are approximate settings, and may require further adjustment.

Fit a glass square made by the balancedbreak method into the front guide fork. Ensure that the square is aligned in the standard orientation used in the laboratory, that is, with the edge chosen to form the knife on the right side and with the score marks downwards (Fig.45). Engage the back guide fork.



Fig.45 Alignment of square

4. To set the tension on the back guide fork. loosen the locking screw, then push the graduated plate inwards to touch the square. Push the back guide fork away from the square into the disengaged position, then move the graduated plate towards the square by 2mm. Secure in this position with the locking screw.

Engage the back guide fork and fit the retrieving fork under the square.

Lower and clamp the scoring head.

7. Score the glass but do not break it. Raise the scoring head and disengage the back guide fork. Use the retrieving fork to remove the square.

Examine the depth of the score immediately. The score should be light and of an even depth over its entire length (Fig.46). If the score is incomplete or too faint, increase the scoring pressure. If the score is too deep and glass splinters can be seen beside the score. reduce the scoring pressure. Refer to Section 9.5 for instructions.

NOTE: The score deepens gradually and if examined more than about five minutes after scoring it may appear too deep. Therefore, it is recommended that the score depth should be examined immediately after scoring.

9. Examine the position of the score on the

glass. If either the short or the long symmetrical scores have been used, they should run centrally over the square and be equidistant from each corner (Fig.47). Measure the distance from the end of the score to the corner (the free break) at both ends of the square. If the two measurements are not the same, adjust the front graduated fork accordingly.



Fig.47 Position of the long score

If the traditional medium score has been used. the score can never be symmetrical. However, the distance between the end of the score and the front corner of the square should be 1mm or less. If it is not less than 1mm, adjust the front graduated fork (Fig.48).

Make an equal adjustment in the same direction to the back graduated plate.



Fig.46 Correct depth of score



Fig.48 Position of the traditional score

 Insert another square into the KnifeMaker and make another score. Examine this score and if satisfactorily, proceed to make the further adjustments.

7.3 Adjusting the counterpiece width

1. Insert another square, lower the scoring head, then score it with the same score length. This time gently raise the breaking handle until the glass fractures.

NOTE: It is recommended that these adjustments are made without using the damping pad since the shape of the knife may be inconsistent if the pad is positioned incorrectly. When correctly applied, the damping pad extends the useful length of knife edge, but makes no improvement to the shape of the knife.

2. Raise the scoring head. Disengage the back guide fork and remove the two knives carefully on the retrieving fork.

3: Without disturbing their relative positions, examine the near corner. For routine sectioning in ultramicrotomy and histology, the knife should be formed to the right of the corner. Examine the width of the counterpiece formed opposite this knife. For a square broken by the balanced break method, this should be 0.4mm or preferably less (Fig. 49).



4. The width of the counterpiece is adjusted by moving the corner of the square laterally. using the counterpiece adjustment controls. An arrow indicates the direction of movement. First loosen the thumb screw on the counterpiece adjustment control, then turn the control using a little downwards pressure. To increase the width of the counterpiece, the corner of the square should be moved to the left by turning the counterpiece adjustment control anticlockwise. To reduce the width of the counterpiece, the corner of the square should be moved to the right by turning the counterpiece adjustment control clockwise (Fig.50). Make an adjustment of 2 divisions in the direction required, then relock the screw.



Fig.50 Counterpiece width adjustment

5. Insert another square and score and break as before. Examine the width of the counterpiece as before and make any further adjustment necessary.

6. When the width of the front counterpiece is satisfactory, examine the back counterpiece. This should be the same width as the front counterpiece, showing that the fracture is running symmetrically across the glass. Loosen the thumb screw, and adjust the back counterpiece adjustment control in the direction required as indicated by the arrow.

7. Score and break a further square to ensure that both counterpieces are equal in width. Check also that the knife edge is straight.

8. Once the counterpiece adjustment is complete, it is recommended that the locking screws supplied with the KnifeMaker II are used to secure the adjustment (Fig.51).



Fig.51 C2 adjustment security lock

7.4 Hints for making large angle knives from a rhombus

When making knives of angles larger than 45°, always ensure that the alignment of the glass strip and the rhombus in the KnifeMaker is correct, as described in chapter 6, section 2. The knife should always be formed on the original edge of the strip (Fig.52).



Fig.52 Alignment of the rhombus

Adjusting KnifeMaker II for larger angles of knife follows the same principles as for 45° knives. However, if KnifeMaker II is already set for making 45° knives, some further adjustments will be necessary. To allow for the wider angle of the rhombus, the position of the front and back guide plates must be changed. Adjust the front graduated plate first by moving it towards the operator, say from Jomm to 11mm on the scale. Adjust the back guide plate by the same amount in the same direction.

Make a test score using the assymetrical traditional score (Fig.53) and check that the score is correctly positioned and ends about 1mm from the corner (see 7.2).



Fig.53 Score indicator

Break a thombus into two knives and examine the counterpiece width. Since the rhombus cannot be made by balanced breaking, the freshly fractured edge will show some signs of stress and the corners may not be completely regular. If this is the case, the knife edge may be straighter when the counterpiece is slightly larger than the 0.4mm recommended for a square. Instructions for adjusting the counterpiece are given in 7.3.

7.5 Hints for making small angle knives from a rhombus

When making knives of angles smaller than 45°, always ensure that the alignment of the glass strip and the rhombus in the KnifeMaker is correct, as described in 6.2, Routine Knifemaking. The knife should always be formed on the original edge of the strip (Fig.54).

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Fig.54 Alignment of the rhombus

Adjusting KnifeMaker II for smaller angles of knife follows the same principles as for 45° knives. However, if KnifeMaker II is already set for making 45° knives, some further adjustments will be necessary.

To allow for the narrower angle of the rhombus, the position of the front and back guide plates must be changed. Adjust the front graduated plate first by moving it away from the operator by about 1mm on the scale. Adjust the back guide plate by the same amount in the same direction.





Make a test score using the symmetrical long score (Fig.55), and check that the score is correctly positioned and is equidistant from each corner (see 7.2.9).

Break a rhombus into two knives and examine the counterpiece width. Since the rhombus cannot be made by balanced breaking, the freshly fractured edge will show some signs of stress and the corners may not be completely regular. If this is the case, the knife edge may be straighter when the counterpiece is slightly larger than the 0.4mm recommended for a square. Instructions for adjusting the counterpiece are given in 7.3, but the operator should be aware that producing a good knife from the narrow corners of a rhombus is timeconsuming and requires some patience.



8.1 Introduction

The routine production of ultrathin frozen sections is dependant on many criteria, one of the most important being the quality of the knife edge. As in the sectioning of resinembedded specimens, the sections produced will always be poor if the knife is of inferior quality. Knife Maker II, with the balanced break principle, makes the production of high quality knives for cryoultramicrotomy a standard procedure.

Most researchers agree that the 45° angle is the most suitable knife for cryoultramicrotomy, since it has been found to have the necessary strength and sharpness. Additionally, by using the balanced break method, a knife with a real angle extremely close to the scoring angle can be produced routinely. By keeping the counterpiece smaller than 0.1mm, a real angle of about 47° will result from a scoring angle of 45°.

8.2 Hints for making cryoknives

Since freshly fractured surfaces produced by the balanced break method are plane, these should always be used to form the knife edge. Therefore the square should be inserted into the KnifeMaker II with the fresh fracture to the right of the near corner (Fig.56).



For making knives for cryoultramicrotomy, the operator has the choice of two score lengths. The short score has a long free break, and can give knives of the highest quality. However, since the free break is so long, reproducibility is rather low. The long score has a short free break, and gives knives of good quality. Additionally, because the free break is short,the reproducibility is high.

8.KNIFEMAKING FOR CRYOULTRAMICROTOMY

> The position of both scores on the square is important. To ensure that the break will run symmetrically over the square, the score should be equidistant from the two corners, that is the length of free break should be the same at each end of the square. Careful adjustment of the front guide fork will achieve this. The back guide fork should then be adjusted by the same amount in the same direction, or reset to give the correct tension to hold the glass in position. Full instructions for this adjustment are given in 7.2.

To achieve a real knife angle close to the scoring angle, the counterpiece of the complimentary half of the square should be extremely narrow, less than 0. Imm, if possible. This requires very accurate and fine adjustment of the C_2 adjustment controls so it may be necessary to spend some time setting the controls. It is advisable to use the locking screws to secure the C_2 controls and note the values once the adjustment is satisfactory. When the adjustment is complete, high quality knives can be broken routinely from the squares.

It is important to note that, as the width of the counterpiece is extremely small, the damping device should not be used since it may come into direct contact with the knife edge and destroy it.

8.3 Knife edge quality

All knives should be checked under a stereomicroscope using darkfield and brightfield illumination to assess edge quality and useful length. It should be noted that, as the aim is to direct the break almost into the corner, some knives may be produced on the "wrong" side, that is, to the left of the corner. These knives are usable, providing the operator uses the right side of the edge instead of the left side normally used (Fig.57).



Fig.57 A knife formed to the left of the corner

After selecting knives of suitable quality, they can be coated with a thin layer of tungsten^{*}. This coating has the effect of reducing the friction of the cutting process and lengthens the life of the knife edge. However, tungsten coating of poor quality knives does not give any improvement of performance.

Knives treated with tungsten coating can be used for several days providing mechanical damage to the edge is avoided. It has been found that a simple rinse in running water followed by distilled water and drying with a jet of compressed gas, such as Freon, cleans the knife to allow it to be used for further sectioning.

It should be noted that cryoknives are not recommended for the sectioning of resinembedded specimens since they do not have the same edge strength at room temperature as they have at low temperature. For resinembedded specimens, knives broken with larger complimentary counterpieces, and thus stronger edges, are recommended, as plastics tend to be harder than well-frozen samples.

* Roberts I., J. Microscopy, 103, 113, 1975

9. LOOKING AFTER YOUR KNIFEMAKER II

9.1 Introduction

Replacement parts, such as scoring cartridges, and damping pads, will be required to keep the KnifeMaker II in good working order. Part numbers for re-ordering these spare parts from LKB are listed in Appendix 2. It is recommended that the user maintains a small stock of these spare parts ready for use when required.

9.2 Keeping your KnifeMaker II clean

It is advisable to keep the KnifeMaker II clean and free of any small picees of glass which could cause misalignment of the strip or square. Loosen the wing nut and remove the strip holder plate. Clean under this plate regularly since any glass particles present will scratch and damage the stainless steel cover plate. Use a small brush to clean around the breaking area. All glass particles should be brushed off the instrument, collected and disposed of safely. Although the breaking mechanism is protected from dust and dirt by the stainless steel cover, avoid brushing glass particles into the cavity surrounding the breaking pins.

The stainless steel cover plate and the painted areas of the instrument can be cleaned with a soft antistatic cloth.

9.3 Maintaining the clamping mechanism

As the clamping handle is operated, the scoring head lowers on to the glass under its own weight. Other than gravity, no force is applied to the scoring head, therefore the sliding surfaces must be kept extremely clean. The internal surfaces must be kept clean to allow the clamping mechanism to function correctly.

It is reasonable to expect that all these surfaces will gradually become dirty and will require cleaning. To remove the scoring head, position the clamping handle in the rear position, with the scoring head raised (Fig.58). Gently pull the scoring head up and remove it from the clamping head. Clean the sliding surfaces of the scoring head and the clamping head with a clean cloth dampened with acctone, then replace the scoring head. It is important that these surfaces are kept clean and free of oil or grease. Do not attempt to lubricate the sliding surfaces or any part of the clamping head with oil or grease since this will retain dust particles and may cause inadequate clamping of the glass during breaking.



9.4 Adjusting the locking position of the clamping handle

Bringing the clamping handle from the rear position to vertical, lowers the scoring head on to the glass. When the handle is moved further from vertical to horizontal where the movement tightens, the scoring head is pulled inwards to lock into the clamping head. No further downward movement occurs. It is therefore unnecessary to apply excess pressure on the clamping handle when it is firmly in the horizontal locked position. In general, if the clamping handle is difficult to unlock, then excessive pressure applied will only cause unnecessary wear on the clamping mechanism. The normal position of the clamping handle when locked is horizontal, that is 70 \pm 10mm from the stainless steel cover plate. With continuous use, the locking position of the handle will become lower. Eventually, clamping will be impossible because the handle will touch the stainless steel cover plate. When the clamping handle locks some distance below horizontal, adjustment of the locking position is necessary.

With the scoring head in position on the clamping head, loosen the drop screw under the front surface of the clamping head (Fig.59). Position the clamping handle in the correct horizontal locked position. Fit any Allen key into one of the holes in the adjusting sleeve, and turn the adjusting sleeve by a small distance towards the front of the instrument to tighten it. Hold the adjusting sleeve in this position with the Allen key, then loosen the clamping handle. Relock the handle and check that the locking position is correct. If necessary, make a further small adjustment to the adjustment sleeve, then check the locking position of the handle again. Several small adjustments on the sleeve may be necessary to obtain the correct locking position for the clamping handle. Finally, when the correct position is found. lock the drop screw.

If it is found that this adjustment has to be performed many times during one year, it is advisable that you should check the pressure being applied to the handle by all users in the laboratory.



Fig.59 Adjustment of the clamping handle

9.5 Adjusting the scoring pressure

To check the scoring pressure, insert a square and use the traditional medium score to score the glass. Remove the square without breaking it, and examine it immediately. The score should be clearly visible across the square. It should be light, but of an even depth (Fig.60).



Fig.60 Correct depth of score

If the score is too deep or too light, move the clamping handle to the back position, and remove the scoring head. Turn the head upside down and insert the Allen key supplied into the pressure adjusting screw (Fig.61). If the score is too deep, turn the screw anticlockwise about half a turn. If the score is too faint, turn the screw clockwise about half a turn. Replace the head and make another test score. Adjust further if necessary.



Fig.61 Adjusting the scoring pressure

9.6 Replacing the scoring wheel cartridge

The scoring wheel cartridge in KnifeMaker II contains the scoring wheel and wheel shaft in one unit. When the score becomes uneven or faint and cannot be corrected by adjusting the scoring pressure, the cartridge should be replaced.

Move the clamping handle to the back position, and remove the scoring head. Turn it upside down and pull out the scoring shaft slightly until the locking screw is accessible (Fig.62). If necessary, the pressure mechanism can be lifted slightly to access the screw. Unscrew the drop screw, and lift out the old scoring wheel cartridge. Insert a new cartridge with the round side facing the drop screw, then carefully tighten the drop screw.



Fig.62 Replacing the scoring wheel cartridge

With a new scoring wheel, it will be necessary to reduce the scoring pressure with the adjustment screw. Replace the scoring head and make a test score. Adjust the scoring pressure further, if necessary. A scoring wheel should last for approximately one year, however in laboratories with a heavy demand on the KnifeMaker II, it may be necessary to change the wheel more often.

9.7 Replacing the strip holding plate

The strip holding plate is manufactured from a selected plastic material which will not cause any damage to the glass strip. Since the plastic is softer than glass, it will gradually become worn. Whenever the strip holding plate shows signs of wear, or if the glass strip is not being held in the correct position, the strip holding plate should be replaced. Two spares are provided with the instrument, and the part number for re-ordering is listed in Appendix 2.

Loosen and remove the locking nut and washer, then remove the glass strip holding plate (Fig.63). Fit the new one in the same way.



Fig.63 Replacing the strip holding plate

9.8 Replacing the damping pad

After some time, the damping pad will become worn and damaged, and should be replaced. This pad is in frequent contact with the corner of the glass square therefore small pieces of glass can become embedded in it, or it may simply become worn and less effective.

If the damping pad no longer improves the knife edges, or if it appears to be damaged, it should be rotated to present a new side to the glass corner. Record the setting on the front graduated plate, then loosen the locking screw and remove the damping device. Push the damping pad slightly out of the holder with the thumb nail and rotate it about a quarter turn. Push it back into position so that it is level with the top of the semi-circular damping pad holder (Fig.64). Replace the damping device and reset the graduated plate to the original reading.



Fig.64 Height of the damping pad

Alternatively, if the entire surface of the damping pad has already been used, push out the old pad and fit a new one. Ensure that it is level with the top of the semi-circular damping pad holder.

9.9 Replacing the white teflon rings

The ends of the damping device are fitted with circular white teflon rings which protect the corner of the glass (Fig.65). These teflon rings may become rough and uneven after some years of constant use, and will no longer hold the glass square or rhombus in the correct position. It is advisable to check these feet routinely, and rotate them when they show signs of wear. Eventually, when they are completely worn out, they should be replaced.



Fig.65 White teflon rings

TECHNICAL SPECIFICATIONS

APPENDIX 1

Methods of operation:	selectable: traditional (sequential) or balanced break
	glass knives for ultramicrotomy, histology and cryoultramicrotomy can produce two knives from each 25 x 25mm square
Scoring Angles of knives:	35°, 37.5°, 40°, 42.5°, 45°, 47.5°, 50°, 52.5°, 55°
Sizes of strips accepted:	thickness: up to 10mm length: up to 400mm
Scoring wheel:	tungsten carbide, mounted in a holding cartridge
Scoring lengths:	four separate lengths in preset positions, one for scoring the strip, and a choice of three for scoring the square or rhombus.
Dimensions:	L x W x H: 480 x 300 x 340mm
Net weight:	16kg
Packed weight:	24kg

ACCESSORIES AND SPARE PARTS

APPENDIX 2

LKB 7890-04	Glass strips for ultramicrotomy, 400 x 25 x 6.4mm (ptk/30)
LKB 7890-08	Glass strips for ultramicrotomy, 400 x 25 x 8mm (pkt/24)
LKB 2078-258	Glass strips for histology, 400 x 25 x 8mm
LKB 2128-560	Knife storage box (pkt/1)
0 02 1733	Scoring wheel assembly complete (pkt/1)
94 91 1783	Damping pad (pkt/5)
0 01 9266	Glass strip holder (pkt/1)
94 91 1839	White teflon ring (pkt/1)