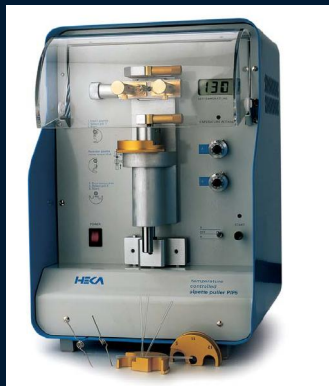


Manual 1.0



**PIP 5**

Pipette Puller



**HEKA**

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Title Page: Temperature Controlled Pipette Puller PIP 5

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# 1. Safety Guidelines

Please read the instruction manual of the PIP 5 before taking the puller into operation to prevent any possible damage to life and equipment. In addition to the instruction manual of the PIP 5 puller, the regulations of prevention of accidents applicable to your country (VGB 4 in Germany) and the relevant rules for safety of the working environment are applicable.

The instruction manual has been designed such that taking the PIP 5 pipette puller into operation is comprehensible, safe, economical and helps to prevent dangerous misuse. A safe use of the puller, minimal service costs and no delay in service can be guaranteed only if the instructions given in the operation manual are being followed. The instruction manual should always be in proximity to the puller. Misuse, neglected inspection of the instrument or disregarding operating instructions may endanger user/s or any third party and may cause damage to technical equipment.

The PIP 5 pipette puller is manufactured according to currently applicable safety regulations. The puller is to be operated only if it is working properly. The puller should immediately be sent for repair if any technical problem occurs which may endanger the safety of the user/s or any third party. The PIP 5 is only to be used for its intended purpose as described in the instruction manual. "Intended purpose" includes regular inspection and service of the puller.

It is possible to add technical equipment to the puller. This equipment is not defined as an "instrument" according to European Community (EC) rules. Thus, equipment can only be added if it is labeled with the CE-certification and has an accompanying statement certifying conformity with EC-rules.

Only technical equipment approved by HEKA can be added to the puller. Information concerning this matter will be provided on request by our service team. Any further use of the PIP 5 and added equipment, which does not fall within the "intended purpose" of the puller, is not in accordance with the liability regulations. HEKA does not accept liability for any dam-

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age caused by misuse of the PIP 5. Manipulations of the instrument are not permissible and lead to loss of liability by the manufacturer.

Please contact HEKA before taking the PIP 5 puller into operation if you are uncertain regarding operating instructions, safety rules or the instruction manual in general.

The PIP 5 instruction manual does not provide instructions for repair. Any necessary repair of the puller has to be done by certified specialists. HEKA offers such certified service.

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## 2. Introduction

The PIP 5 is a temperature controlled puller which is a further step to make pulling of glass pipettes easier and more comfortable in the sense of a better reproducibility. It was developed for pulling glass pipettes as they are used for clamp experiments. The range of application of the produced pipettes covers e.g. intracellular recording pipettes or patch-clamp pipettes. The pipettes are being produced by a 2-step vertical pull at different temperatures and pull lengths. Due to the symmetry of the pull you will always receive two equivalent pipettes.

Special features:

- temperature controlled heating filaments
  - high filament temperature range: 300 ° C - 1900 ° C
  - precise temperature control providing a high reproducibility of pull procedures
  - independent filament temperatures for first and second pull
  - heating filament adjustable into two dimensions
  - suitable for all types of soft and hard glass (except Quartz glass) of 1,3-3 mm diameter
  - faster attained set temperature especially with the second pull
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## 3. Operation

Two pipettes are being pulled from a glass tube. The shape of the pipette tip is determined by the time of pull, temperature and the glass type to be used. Usually the tips have a diameter of 1-2 $\mu$ m.

In the following the steps will be described you need to pull pipettes with the PIP 5. Also some examples of typical pipettes are given.

### 3.1 How to pull your pipettes

1. Insert the glass tubes into the guidance and fix the upper end.
  2. Move the pull mechanism to the upper position and fix the lower end of the glass tube with the tension screw.
  3. Fit the spacer disk onto the guide shaft and turn counterclockwise until stop position.
  4. For the first pull select the desired heating filament temperature at potentiometer A, set temperature selection switch to position A and press the Start button.
  5. The heating filament starts to glow and melts the glass tube in its center. The tube is being pulled by gravitation. At the end of each pull the heating filament will be switched-off automatically.
  6. Loosen the upper tension screw, lift the pull mechanism, turn the spacer disk clockwise until stop position and lower the pull mechanism again until it sits on the spacer disk.
  7. Tighten again the upper tension screw.
  8. Now turn the spacer disk to medium position and start the second pull procedure: select the desired heating filament temperature at
-

disk number	A (mm)	B(mm)	1st pull length (mm)
7	9.0	13.5	7.0
9	7.0	12.8	9.0
11	5.0	12.0	11.0

potentiometer B, set the temperature selection switch to position B and press the Start button.

- With the second pull the glass breaks and two pipettes with identical tips are produced.

### 3.2 How to select the right spacer disk

The shape of the pipette is amongst others determined by the length of the first and second pull. For that reason our delivery volume includes three spacers disks which allow the pull of different types of pipettes (see figure 1). The disk number determines the length of the first pull (16mm-A, see: Adjustment of the pulling mechanism). Distance B serves for identical tips.

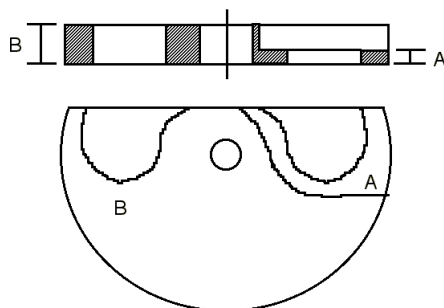


Figure 3.1: Spacer disk with distances A and B

With a first long pull (disk# 11) you will receive pipettes with long thin tips. Short tips with a low electrical resistance will be received with short pull lengths e.g. with disk # 7.

## **3.3 Examples**

### **3.3.1 Patch pipettes**

1. Kimax 51 Borosilikat glass, first pull  $1200^\circ$  , second pull  $765^\circ$  , with spacer disk no. 7 leads to pipettes with a resistance of 3 MOhm.



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## 4. Adjustment of PIP 5 pipette puller

The pulling mechanism is preset before delivery. In case there will be a change of settings (e.g. an exchange of the heating filament) the pulling mechanism and the heating filament can be easily adjusted by yourselves.

### 4.1 Adjustment of the heating filament

Insert a heating filament into the filament receptor, however do not tighten the tension screws (lower side of the support).

Push the glass tube through the heating filament and tighten it by means of the tension screws. Turn the heating filament into horizontal position and fix it (lower side of the support). Adjust the heating filament with the adjustment screws in such a way that the glass tube will be in centric position (screws at front side of the support resp. thumb-wheel left of the support).

### 4.2 Adjustment of the pulling mechanism

For adjustment please use the adjustment block (16 mm) as well as the hexagon wrench which is included with the delivery.

1. Switch-off the puller
  2. Remove the lower cover.
  3. Adjust the vertical movement of the pulling mechanism to 16 mm by means of screw 2 (figure 1). Please use the 16 mm adjustment block for that purpose.
-

4. By means of screw 1 (figure 2) adjust the left gear bar in such a way that it will operate the limit switch as per figure 3a. (The pulling mechanism is at lowest position).
5. Adjust the right gear bar according figure 3b by using screw 3 (figure 2). (The pulling mechanism is at lowest position.)
6. Mount the lower cover.

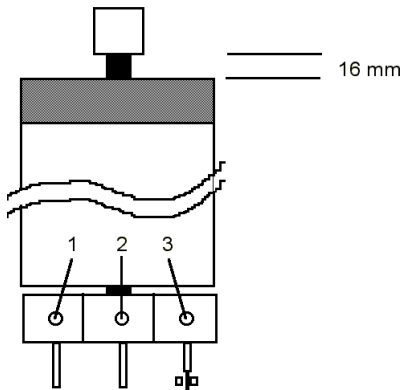


Figure 4.1: Pulling Mechanism

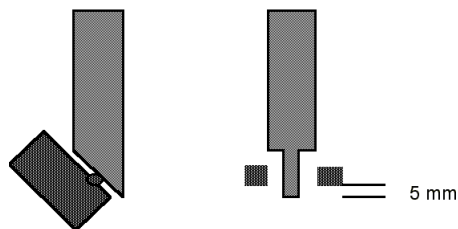


Figure 4.2: End Switches

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## 5. Appendix

### 5.1 Glass types

The following two tables show some glass types and the supplier:

<b>Soft Glass (<i>Supplier</i>)</b>	<b>OD</b>
Non-heparinized hematocrit tubing <i>any scientific supplier</i>	1.3 mm
Drummond Microcaps <i>Drummond Scientific, Bloomall, PA, U.S.A.</i>	1.4 mm

<b>Hard Glass (<i>Supplier</i>)</b>	<b>OD</b>
Kimax 51 <i>Kimble Products, Vineland, NJ, U.S.A.</i>	1.7 mm
Boralec 100 <i>Rochester Scientific, Rochester, NY, U.S.A.</i>	1.7 mm
Corning Sealing Glass (# 7052, # 7040) <i>Dow Corning, Midland, MI, U.S.A.</i>	1.6 mm
GCASS 150-4 (aluminum glass) <i>A-M Systems, Everett, WA, U.S.A.</i>	1.5 mm

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## 5.2 Literature

The technique of pulling glass pipettes is described in the following articles:

B. Sakmann and E. Neher: *Geometric parameters of pipettes and membrane patches*. In: *Single-Channel Recording* (B. Sakman and E. Neher, eds), Plenum Press, New York

Hamill, O.P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F.J. (1981) *Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches*. Pflügers Arch. **391**, 85-100.

Rae, J. & Levis, R. (1984) *Patch clamp recordings from the epithelium of the lens obtained using glasses selected for low noise and improved sealing properties*. Biophys. J. **45**, 144-146.