

Multiporator®

***Bedienungsanleitung
Operating Manual
Mode d'emploi
Istruzioni d'impiego
Manual de Instrucciones***



eppendorf



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1 General information

The Multiporator® is designed for the electroporation of eukaryotic cells. With the aid of optional modules, it is also possible to perform electroporation on bacteria and yeast as well as fusion of cells. This manual contains information on all possible uses of the Multiporator®.

The outstanding feature of the Multiporator® is that the parameters entered for the electroporation of eukaryotic cells and for cell fusion are maintained exactly by means of internal calculation of the discharge curve.

2 Intended Use

The Multiporator is designed for controlled, reproducible electroporation of eukaryotic cells. Precise regulation of the discharge curve controls the reproducible opening of the cell membrane and cell-preserving intake of biomolecules.

The device is only intended for use in research laboratories. It may only be operated by qualified lab personnel. The Multiporator can be optionally equipped with modules for cell fusion and electroporating bacteria and yeasts.

3 Safety precautions

For reasons of personal safety, it is essential to observe the following!

- **Observe the instructions contained in the operating manual**
It is essential to comply with the information given in the manual!
- Only use the device with a power supply which has a grounding contact!
- Before starting up the Multiporator[®], please ensure that the power supply used corresponds to the voltage specifications on the identification plate!
- Do not use the device if it is damaged, especially if the mains cable is damaged.
- The unit may be opened only by authorized service personnel! Before opening the device, switch it off and remove the power supply plug. Potentially lethal voltage inside the unit.
- Do not operate the device in an explosive environment!
- Do not allow any liquid to enter into the Multiporator[®] or the inserts!
- The device should be used for *in vitro* applications only!
- Use the device only for the purpose for which it is intended.
Do not place parts, devices, tools or other objects into the cuvette insert which have not been recommended by Eppendorf AG.
High voltage!
- Use the listed accessories only.
Other accessories may only be used if they are accompanied by a safety certificate from Eppendorf AG confirming their suitability for use!
- Electrical connection to devices which are not mentioned in this operating manual is only permitted with the prior consent of the manufacturer!
- The Helix fusion chamber must be handled with care.
Loose or damaged electrodes adversely affect operating procedures.
In such cases, the Helix fusion chamber must not be used!
Do not open the Helix fusion chamber until fusion has finished!
- The Micro fusion chamber must be handled with care.
Take care when filling with cell suspension. Do not touch or move the electrodes with the pipette tip! This may cause damage.
During cell alignment/fusion, avoid direct contact with the electrodes!

3 Safety precautions

- Cell alignment/fusion may be performed only when the Micro fusion chamber is connected.
The cable is designed for connection to the Micro fusion chamber only. No other connections are permitted! Do not place the plug into liquid!
- The owner or operator of the device is liable for the functioning of the device when the device is serviced or maintained by persons who are not members of the Eppendorf service personnel or when the device is not operated in accordance with the given regulations and precautions. Eppendorf AG accepts no liability for damage resulting from such action. Warranty and liability stipulations which form part of the terms of sale and delivery of Eppendorf AG are not affected by the above-mentioned conditions.
- This operating manual or parts of this operating manual may not be reproduced in any form without the prior written permission of Eppendorf AG!
- Eppendorf AG reserves the right to make technical alterations to this product!
- **Transfer**
If the device is passed on to someone else, please include the instruction manual.
- **Disposal**
In case the product is to be disposed of, the relevant legal regulations are to be observed.
- **Information on the disposal of electrical and electronic devices in the European Community**
The disposal of electrical devices is regulated within the European Community by national regulations based on EU Directive 2002/96/EC on waste electrical and electronic equipment (WEEE).

According to these regulations, any devices supplied after 13.08.05 in the business-to-business sphere, to which this product is assigned, may no longer be disposed of in municipal or domestic waste. They are marked with the following symbol to indicate this.



As disposal regulations within the EU may vary from country to country, please contact your supplier if necessary.

4 Device description

The device has four different modification levels: the basic device is used for the electroporation of eukaryotic cells. It can then be developed for the electroporation of bacteria and yeast, with an additional level offering the opportunity to perform cell fusion. These two levels can be combined to form the fourth level.

The Multiporator® is operated using an easy-to-follow keypad with display. The insert for the electroporation cuvette is located next to the keypad.



Fig. 1: Multiporator®, front view

4 Device description

4.1 Delivery package

Basic device: Multiporator® for the electroporation of eukaryotic cells; with mains cable
Insert for electroporation cuvettes
Electroporation cuvettes
(Electrode gap width: 2 mm / 4 mm)
Operating manual and Basic Applications Manual

"Bacteria module" optional:
Basic device incl. accessories
with integrated bacteria module
Additional electroporation cuvettes
(Electrode gap width: 1 mm)

"Cell fusion module" optional:
Basic device incl. accessories
with integrated cell fusion module
Helix fusion chamber
(Electrode gap width: 0.2 mm)
Insert for Helix fusion chamber
Micro-fusion chamber
(Electrode gap width: 0.2 mm) with special insert

"Fusion- and bacteria module" optional:
Basic device incl. accessories
with integrated fusion- and bacteria module.

4 Device description

4.2 Startup

The Multiporator must stand completely on the stable work surface and be safely positioned. There must be enough space that the front and rear ventilation slits are not covered and that air for cooling can reach underneath the device.

Space requirement: Width: 25.2 cm
Depth: 34.2 cm
Height: 12.0 cm

The main power socket with fuse and main power switch is located on the rear of the Multiporator®. The mains power must agree with the information on the ID plate. To start the device, insert the power cable into the main power socket and connect it up to the main power supply. Turn on or off the device by pressing the main power switch.



Fig. 2: Multiporator®, rear view
Power connection (left)
Printer connection (right)

The delivery carton should be kept in order to be able to safely send back the device in case repairs are necessary.

4.3 Cuvette insert

The insert for the electroporation cuvette is next to the keypad at the front of the device. To remove the cuvette, pull out the cuvette insert. When inserting a new cuvette, ensure that the nose of the cuvette fits into the rear slot. Push the cuvette insert containing the cuvette into the device up to the stop. A sensor in the device detects whether the cuvette insert contains a cuvette.

Note: When transporting the device, please ensure that the insert contains an empty cuvette. Alternatively, transport the cuvette insert separately.

Different inserts are used for the modification level "cell fusion".

4 Device description

4.4 Keypad

The keypad is used to enter the electroporation or the electrofusion modes, to select the parameters and to start the application. Depending on the modification level, 1 to 3 modes can be selected. Key combinations can be used to set the date and time as well as the volume of the acoustic signal.

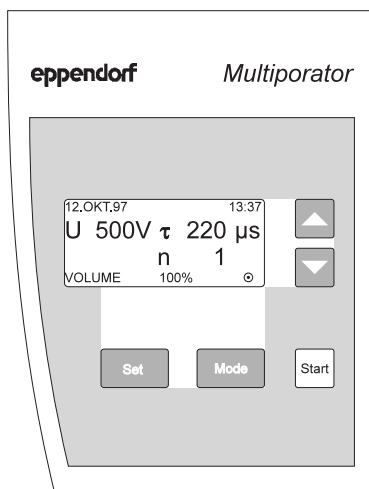


Fig. 3: Control panel with keys and display

4.5 Keys and key combinations



SET

The SET key is used to select the desired parameter. This parameter is underlined in the display. Further parameters are selected by pressing the SET key an appropriate number of times. A SET sequence is ended either by pressing the SET key repeatedly until the underlining disappears or by pressing the MODE or START key.

(Note: The MODE key switches to another mode if available.) Parameters are modified using the up/down arrow keys. When the lowest possible value has been reached, the display then moves to the highest value. Similarly, when the highest value has been reached, the display then moves to the lowest value.

4 Device description

A grey rectangular button with the word "Mode" in white text.

MODE

The MODE key is used to activate the mode with which electroporation or electrofusion is to be performed. It is only possible to select modes in accordance with the modification level of the device. This means for the basic device, the mode for the electroporation of eukaryotic cells is available, while there is a choice of three modes for the maximum expansion level.

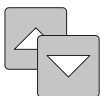
The corresponding symbol then appears in the display:

- ⊙ : Mode for eukaryotic cells
Modification level: Basic device
Pulse used: Decaying e-function
- : Mode for bacteria and yeasts
Modification level: Bacteria module
Pulse used: Decaying e-function
- ☺ : Mode for cell fusion
Modification level: Fusion module
Pulse used: Square-wave pulse

A white rectangular button with a black border and the word "Start" in black text.

START

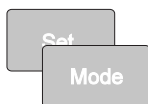
The process required is started by pressing the START key.



Arrow keys

The arrow keys are used to modify the selected (i.e. underlined) parameters in the desired direction.

4 Device description



Setting the date

The procedure for setting the date and time can be activated by pressing the SET and MODE keys simultaneously. If the SET key is pressed again, the underlining in the display for the date and time moves from left to right. The arrow keys can thus be used to modify the settings.

To end the process for setting the date, press the SET key repeatedly until the underlining is no longer visible in the display. Alternatively, the underlining disappears from the display when the MODE or START key is pressed. (**Note:** The MODE key switches to another mode if available.)



Setting the volume of the acoustic signals

The procedure for setting the volume of the acoustic signals can be activated by pressing the MODE and START keys simultaneously. "VOLUME" then appears in the bottom line of the display, together with a figure in percent. This figure indicates the volume and the tone type and can be modified using the arrow keys.

To end the procedure for setting the volume of the acoustic signals, press the SET, MODE or START key. (**Note:** The MODE key switches to another mode if available.) "VOLUME" then disappears from the display.

Reset

An experiment can be terminated only by pressing the main power switch. This switch also functions as a reset switch.

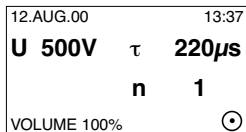
4 Device description

4.6 Display

The date and time of day are always displayed in the uppermost line of the display. The parameters which are relevant for the experiment appear in lines 2 to 4 in larger letters and in bold print. The parameters and the number of parameters differ according to the mode which has been selected. The mode which has been selected is always represented by a symbol on the right of the bottom line:

- ☉ : Mode for eukaryotic cells
Modification level: Basic device
- : Mode for bacteria and yeasts
Modification level: Bacteria module
- ☺ : Mode for cell fusion
Modification level: Fusion module

"VOLUME" or the remaining time for the experiment can also appear in the display in this line.



Example of the display for setting the volume of the acoustic signal

During and after the experiment, the display changes to show the information relevant to the stage of the experiment which has been reached.

4 Device description

4.7 Electroporation

4.7.1 Cuvettes and cuvette insert

The electroporation of eukaryotic cells, bacteria or yeasts is carried out in disposable cuvettes. The biological material is placed in the gap between the electrodes of the cuvette, whereby the prescribed liquid volumes of the relevant cuvette are to be maintained. Although a supernatant above the electrode does not drastically affect the experiment, however it reduces the general efficiency.

A matt window allows the cuvette to be inscribed.

The plastic nose on the cuvette ensures that the cuvette is inserted correctly into the cuvette insert. The lid seals the cuvette. However, to safeguard against excess pressure, the cuvette is not hermetically sealed by the lid. A filled cuvette should therefore be transported in an upright position only in order to ensure that biological material does not leak out.

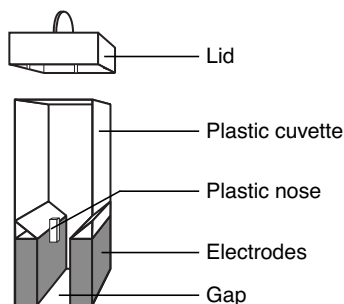


Fig. 4: Schematic diagram of electroporation cuvette

The cuvette insert is removed from the Multiporator[®] using the protruding grip. No bubbles are present when the cuvette is filled.

When the cuvette is inserted into the inner recess of the cuvette insert, care must be taken to ensure that the cuvette nose is positioned in the long slit and that the cuvette is in contact with the base of the cuvette insert. The cuvette insert, complete with cuvette, is then pushed into the device up to the stop.

The cuvette insert may be stored in a cool place.

When transporting the device, please note that the cuvette insert may slip out of the device if no cuvette has been inserted (see chap. 4.3).

4 Device description

4.7.2 Electroporation buffer for eukaryotic cells

To achieve optimal transfection results, the original electroporation buffer from Eppendorf, with a low electrical conductivity, should be used. These electroporation buffers (hypo and isoosmolar) are tested for sterility and the absence of mycoplasmas and endotoxins.

The use of these buffer systems means that considerably less current flows during electroporation, with the result that no significant damage is sustained by the cells. Simultaneously, the use of appropriate buffer media results in the electrically induced "pores" being much larger than those of pulse applications in conductive solutions.

Ideally, the electroporation should be carried out in hypoosmolar buffer. Through the hypoosmolar buffer system, the cells absorb water shortly before the pulse and swell as a result. Due to a variety of effects, including the lowering of the breakdown voltage, this process enables increased permeability of the plasma membrane. The yields can thereby be considerably increased in comparison to those under isoosmolar conditions. In the case of cells that react sensitively to purely hypoosmolar conditions, step-by-step addition of the isoosmolar electroporation buffer can be used to adjust the necessary osmolarity.

The Eppendorf electroporation buffer should be used for the electroporation of eukaryotic cells (with the exception of yeasts and some microorganisms and plants). Electroporation media with low conductivity can be used for the electroporation of bacteria, yeasts and other microorganisms. Detailed application protocols can be downloaded from the Eppendorf Homepage www.eppendorf.com.

4 Device description

4.8 Cell fusion (optional)

4.8.1 Micro fusion chamber (Electrode gap width 0.2 mm)

With the help of the Micro fusion chamber, the parameters for the cell alignment and for the cell fusion can be optimized under microscopic control.

The Micro fusion chamber consists of a housing equipped with a transparent reservoir, into which two electrodes with a gap of 200 μm are embedded. The connection to the Multiporator takes place through a coaxial cable (approx. 1 m) over a special insert (see Fig. 5).

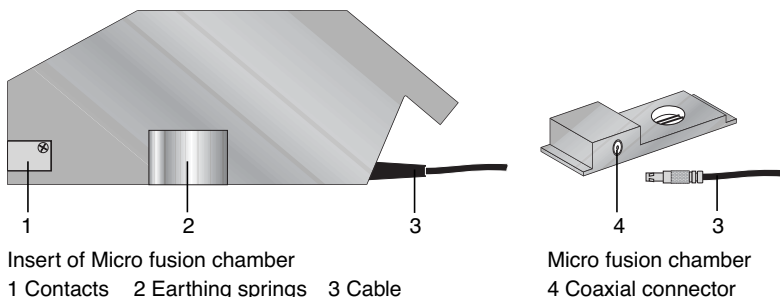


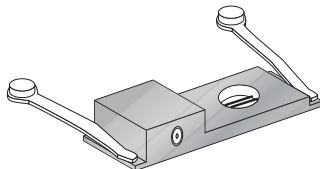
Fig. 5: Schematic diagram of the insert and the Micro fusion chamber

Caution: The Micro fusion chamber must be handled with care. Take care when filling with cell suspension. Do not touch or move the electrodes with the pipette tip. This may cause damage!



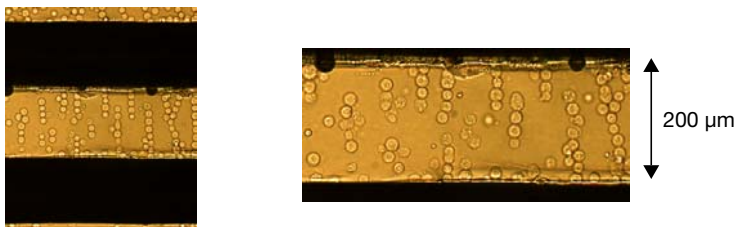
During cell alignment/fusion, avoid direct contact with the electrodes. Cell alignment/fusion may be performed only when the micro fusion chamber is connected.

- Slide the Micro fusion chamber firmly into the Multiporator®.
 - Place the Micro fusion chamber (which is filled with approx. 20–50 μl cell suspension) onto the microscope.
- Note:** Pipetting the cell suspension carefully onto the area around the electrodes can maximize the number of cells between the electrodes.
- The brackets can be used to lock into place on the microscope table. They can also be used to fasten a glass slide.



4 Device description

- Connect the Micro fusion chamber with the coaxial connection of the cable.
- Focus the electrodes and cells under the microscope.
- Run through the parameters by pressing the START key. This can be monitored under the microscope (see chap. 5.3).



Alignment of the cells
between the electrodes

Cells after fusion and fusion products

Fig. 6: Microscopic view of the cells during the alignment and after the fusion sequence with individual fusion products

The parameters determined to be optimal can be directly adopted for the fusion in the Helix fusion chamber (see chap. 4.8.4).

4.8.2 Micro fusion chamber (Electrode gap width 0.5 mm)

The non-standard accessories (see "Ordering information") contain a Micro fusion chamber with a modified electrode interval of 500 μm . With the help of this Micro fusion chamber, cells can be fused under microscopic control.

General instructions for usage of a Micro fusion chamber are given in chapter 4.8.1 and in the Basic Applications Manual for electrofusion. This is included in the delivery package of the fusion module.

Important note: When calculating the fusion parameters (see Basic Applications Manual for electrofusion), the modified electrode interval of 500 μm must be taken into account. Please note that the parameters determined as optimal cannot be adopted directly for fusion in the Helix fusion chamber.

4.8.3 Cleaning the Micro fusion chamber

The content of the Micro fusion chamber is rinsed out using bi-distilled water from a spray bottle. Cell residue that is particularly stubborn can be removed by carefully cleaning the electrodes with a soft toothbrush using vertical strokes. (The space between the electrodes must not be changed during this process!) The drying process can be accelerated by rinsing the chamber with 70 % non-denatured ethanol.

4 Device description

4.8.4 Helix fusion chamber

The Helix fusion chamber has been specially designed for the production of larger quantities of fusion products (hybrids). It consists of a conical tapered core that carries the electrodes and a beaker into which the cell suspension is added (see fig. 7).



Fig. 7: Helix fusion chamber

Diagram of Helix fusion chamber

The Helix fusion chamber (see fig. 8) consists of a cup (1) and a core with parallel wound platinum wires, which are the electrodes (2). They are wound around the core as a double helix. Both parts are screwed together. The gap (4) between the cup and the core has a filling volume of 250 μ l.

The Helix fusion chamber is linked to the insert (6) for cell fusion via the coaxial connector (3).

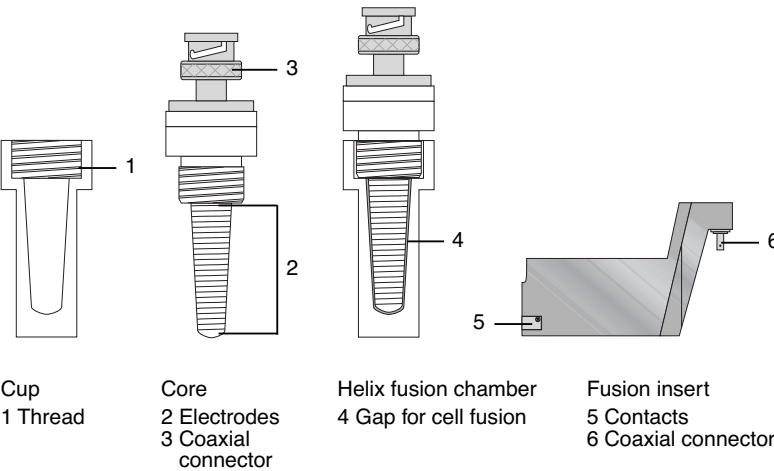


Fig. 8: Schematic representation of the Helix fusion chamber and the fusion insert

4 Device description

The gap between the wound platinum electrodes is approx. 200 μm .

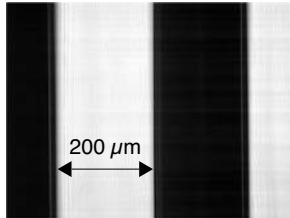


Fig. 9: Microscope view of the electrodes of the Helix fusion chamber

Caution: Handle the Helix fusion chamber with care!
Loose or damaged electrodes adversely affect operating procedures.
In such cases, the Helix fusion chamber must not be used!

4.8.5 Filling the Helix fusion chamber

The Helix fusion chamber is filled with 250 μl cell suspension. It is essential to ensure that the liquid is pipetted into the deepest possible position in the cup. The edge and the walls must not be wetted as this has an adverse effect on filling the Helix fusion chamber. Air bubbles may form which reduce the effectivity of the experiment.

The core is inserted into the upright cup in the stand and carefully screwed into place. As a result of this screwing action, the cell suspension in the ever-decreasing gap is forced upwards.

4 Device description

When screwing the core, make sure that no air bubbles form.

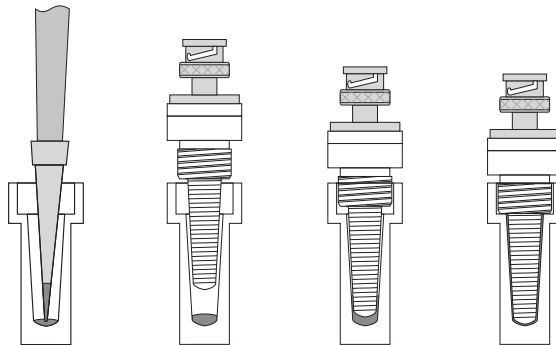


Fig. 10: Filling the Helix fusion chamber (schematic representation)

Leave the closed Helix fusion chamber upside-down on the coaxial connector until the fusion process has been completed!

4.8.6 Fusion and cell suspension extraction

- Slide the fusion insert firmly into the device.
- Place the closed Helix fusion chamber onto the coaxial connector and lock it into place by rotating it a quarter-turn.
- Carry out the fusion with the set parameters (see chap. 5.3).
- Loosen the Helix fusion chamber carefully by rotating it a quarter-turn and then remove it.
- To open the chamber, place it in a vertical position (e.g. in the stand).
- Unscrew the core carefully from the beaker.
- Using a small quantity of the appropriate medium, rinse off any cell suspension that may be on the core.
- The cell suspension from the beaker is mixed with the cell suspension that has been rinsed off and is then treated further as required.

4 Device description

4.8.7 Cleaning and disinfecting the Helix fusion chamber

The beaker and core of the Helix fusion chamber should be rinsed with distilled water directly after the experiment in order to prevent cell and buffer residue from drying up.

If heavily contaminated, the Helix fusion chamber should be cleaned briefly in an ultrasonic bath (possibly with a cleaning supplement, such as Edisonite Super) or with a very soft (tooth)brush. When cleaning is carried out using brushes, please ensure that brushing is carried out in the same direction as the windings, since the electrodes may otherwise move out of their correct position and thus render the Helix fusion chamber unusable.

Disinfect the parts using non-denatured 70 % ethanol. To do this, the beaker is filled with 250 μ l of ethanol and the core screwed into the beaker. After **10 seconds**, the core is unscrewed and the alcohol can be removed. To subsequently dry the beaker and the core place them in the stand under sterile, dust-free conditions. After drying, the Helix fusion chamber may be re-used.

4.8.8 Electrofusion buffer for eukaryotic cells

The electrofusion medium (fusion buffer) is of considerable importance for the survival rate of the cells and for the successful extraction of hybrid cells. Only a single buffer should be used for the entire electrofusion procedure (alignment, fusion, post-alignment) in order to protect the cells from additional stress. The fusion buffers from Eppendorf are differentiated from the commonly used fusion media by low conductivity and low osmolarity. The low conductivity (120 μ S/cm) of the Eppendorf buffers enables the application of relatively low field strengths (voltage) when merging cells.

Ideally, electrofusion should be carried out in hypoosmolar buffer. As a result of the hypoosmolar buffer system, the cells absorb water shortly before the pulse and swell. The membrane and actin skeleton proteins are thereby temporarily disengaged, thus easing fusion in the electrical field. The yields of fusion products attained can thereby be considerably increased in comparison to those under isoosmolar conditions. For cells that react sensitively under purely hypoosmolar conditions, the necessary osmolarity can be adjusted through step-by-step addition of the isoosmolar buffer.

The Eppendorf fusion buffers (hypo and isoosmolar) are tested for sterility and the absence of mycoplasma and endotoxins.

4 Device description

4.9 Insert (electroporation / electrofusion) for connecting external electrodes

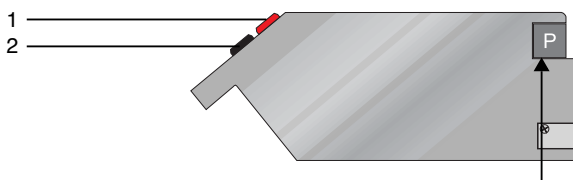


Prior to using the insert for the connection of external electrodes, the supplement sheet accompanying the insert must be read thoroughly. The safety instructions found in the supplement sheet must be observed.

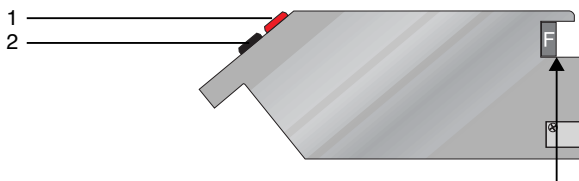
The non-standard accessories (see "Ordering information") contain an insert, to which it is possible to connect external electrodes. By changing the position of a function switch (P/F) accordingly, the insert can be used for electroporation (symbols ○ ⊙) or for cell fusion with external electrodes (symbol ☉).



The electrodes are connected using two 4 mm contact-proof laboratory plugs.



Position of the function switch for electroporation **P**



Position of the function switch for electrofusion **F**

- 1 Connecting socket, positive pole (red)
- 2 Connecting socket, ground (black)

Fig. 11: Insert for the connection of external electrodes (schematic representation)



Attention! Characteristic of electrofusion of cells with external electrodes:

During the electrofusion, a resistance of 50 Ohm must be connected parallel to the electrodes with the help of plug in connections.

4 *Device description*



When external electrodes are connected, the relevant national safety regulations (relating to high voltage) and EMC regulations apply and should be observed (EMC: electro-magnetic compatibility).
The user is responsible for all equipment that has been connected!

5 Mode of operation

5.1 Mode for electroporation of eukaryotic cells

This mode is represented in the display by the symbol ☉.

Instructions for the filling of the electroporation cuvettes have been summarized in chapter 4.7.1 of the operating instructions. A detailed description of the electroporation of eukaryotic cells can be found in the Basic Applications Manual for electroporation, which is included in the delivery package. This application manual can also be downloaded from the Eppendorf Homepage www.eppendorf.com.

Step 1 – Entering / modifying parameters

- If necessary, switch to the mode ☉ by pressing the MODE key.
- Press the SET key to select the parameter desired (the parameter will be underlined).
- Use the arrow keys to modify the parameter in the direction required.
- If necessary, modify other parameters using the SET key and the arrow keys.
- Terminate the entering procedure. The parameters are effective immediately and do not have to be stored.

12.AUG.00	13:37
U 500V	τ 220μs
	n 2
	☉

Example of a display for the electroporation of eukaryotic cells.

The relevant parameters appear in the display in larger letters and in bold print.

U: Voltage, in V (volt).

Can be set in increments of 1 V in the range 20–100 V; in increments of 10 V in the range 100–1,000 V; in increments of 100 V in the range 1,000–1,200 V.

τ : Time constant, in μ s (microseconds).

Can be set in increments of 5 μ s in the range 15–500 μ s.

n: Number of pulses during the experiment. Can be set between 1 and 99. A period of one minute elapses between each pulse.

Note: The course of the curve is calculated internally, so that the course of the discharge curve (e-function) is maintained according to the parameters which have been entered.

5 Mode of operation

Step 2 – Starting electroporation

- Trigger the electroporation process by pressing the START key.
- "Charge" appears in the third line of the display. Charging commences.
- After the charging procedure has ended, discharging occurs. This is indicated by a flash in the third line of the display.

12.AUG.00	13:37
U 500V	τ 220 μ s
Charge	

If more than one pulse has been set, "Wait" appears in the display, accompanied by the remaining waiting time.

12.AUG.00	13:37
U 500V	τ 220 μ s
Wait	
 0:57	

After the experiment has ended, a double acoustic signal is emitted and the initial information appears in the display with the parameters which have been used.

12.AUG.00	13:37
U 500V	τ 220 μ s
n 2	
READY SINCE 1:36	

The time which has elapsed since the end of the experiment appears in the bottom line in minutes and seconds. After 99 minutes or after any key has been pressed, this information disappears from the display.

5 Mode of operation

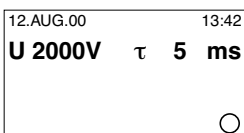
5.2 Mode for electroporation of bacteria and yeast (optional)

This mode is represented in the display by the symbol ○.

Instructions for the filling of the electroporation cuvettes have been summarized in chapter 4.7.1 of the operating instructions. In addition, application protocols for the electroporation of bacteria, yeasts and other microorganisms can be downloaded from the Eppendorf Homepage www.eppendorf.com.

Step 1 – Entering / modifying parameters

- If necessary, switch to the mode ○ by pressing the MODE key.
- Press the SET key to select the Voltage (U) parameter (the parameter will be underlined).
- Use the arrow keys to modify the parameter in the direction required.
- Terminate the entering procedure. The parameters are effective immediately and do not have to be stored.



The display shows the date 12.AUG.00 and time 13:42 in the top corners. The main display area shows the parameter U 2000V, followed by the Greek letter tau (τ) and the value 5 ms. The parameter U is underlined. In the bottom right corner, there is a small circle symbol (○).

Example of a display for the electroporation of bacteria and yeast.

The relevant parameters appear in the display in larger letters and in bold print.


U: Voltage, in V (volt).
Can be set in increments of 10 V in the range 200–1,000 V; in increments of 100 V in the range 1,000–2,500 V.

τ: Time constant, in ms (milliseconds).
Set to 5.0 ms.

5 Mode of operation

Step 2 – Starting electroporation

- Trigger the electroporation process by pressing the START key.
- "Charge" appears in the third line of the display. Charging commences.
- After the charging procedure has ended, discharging occurs. This is indicated by a flash in the third line of the display, accompanied by a double acoustic signal.

12.AUG.00	13:42
U 2000V	τ 5 ms
Charge	
	

After the experiment has ended, a double acoustic signal is emitted and the initial information and the set parameters appear in the display. The actual parameters U_a and τ_a appear in the third line of the display.

The time which has elapsed since the end of the experiment appears in the bottom line in minutes and seconds. After 99 minutes or after any key has been pressed, this information disappears from the display.

12.AUG.00	13:42
U 2000V	τ 5 ms
U_a 2003V	τ_a 5.1 ms
READY SINCE	1:36 

U_a : Actual measured voltage used, in V (volt).

τ_a : Actual time constant used, in ms (milliseconds). Values ≤ 0.8 are shown as 0.8 ms.

5 Mode of operation

5.3 Mode for cell fusion (optional)

This mode is represented in the display by the symbol ☺☺.

Instructions for the usage of the Micro fusion chamber and the Helix fusion chamber have been summarized in chapter 4.8 of the operating instructions. A detailed description of the electrofusion of eukaryotic cells can be found in the Basic Applications Manual for electrofusion, which is included in the delivery package of the fusion module. This application manual can also be downloaded from the Eppendorf Homepage www.eppendorf.com.

5.3.1 Function: Cell alignment

The functions for alignment are indicated by the symbols U'_{\sim} (before the pulse, alignment) and U''_{\sim} (after the pulse, post-alignment) in lines 2 and 4 of the display.

Step 1 – Entering / changing parameters

- If necessary, switch to the mode by ☺☺ pressing the MODE key.

12.AUG.00	13:42
U'_{\sim} 5V	t 60s
U_{\square} 0V	t 15s n2
U''_{\sim} 0V	t 30s ☺☺

Example of the display for cell alignment U'_{\sim} without activation of pulse U_{\square} and alignment U''_{\sim} .

- Press the SET key to select the parameter desired (the parameter will be underlined).

The relevant parameters appear in larger letters and in bold print.

- Use the arrow keys to modify the parameter in the direction required.

If a value in lines U'_{\sim} / U''_{\sim} / U_{\square} are set to "0", the function in question is not carried out. This enables U'_{\sim} / U''_{\sim} to be triggered individually or consecutively without the fusion pulse being activated.

U'_{\sim} / U''_{\sim} :

Alternating voltage, in V (volts). Can be set to "0" and in increments of 0.1 V between 1.0 V and 10 V.

t: Duration of alignment 0–95 s, in s (seconds). Can be set in increments of 5 seconds.

U_{\square} : Voltage, (square-wave pulse) unit V (volts). Can be set to "0" and in increments of 1 V between 5 V and 100 V, and in increments of 10 V between 100 V and 300 V.


5 Mode of operation

- Terminate the entering procedure. The parameters are effective immediately and do not have to be stored.

t: Duration of pulse, Unit μs (microseconds). Can be set in increments of 5 μs between 0 and 15 μs –300 μs .

n: Number of pulses 0–99. Can be set in one-pulse increments.

Step 2 – Starting cell alignment

- Trigger alignment U'_{\sim} by pressing the START key.
- The information  appears in the fourth line of the display; alternating voltage is applied for the duration selected.

12.AUG.00		13:42	
U'_{\sim}	5V	t	60s
U_{\square}	0V	t	15s n2
U''_{\sim}	0V	t	30s

At the end of the experiment, an acoustic signal is emitted and an initial display appears with the parameters that have been set and used.


In addition, the time that has elapsed (in minutes and seconds) since the end of the experiment is shown in the bottom line. This display disappears after 99 minutes or when any key is pressed.

5 Mode of operation

5.3.2 Procedure for cell fusion


Step 1 – Entering / modifying parameters

- Press the SET key to select the parameters desired (the parameter will be underlined).

12.AUG.00	13:42
<u>U'</u> 5V t 30s	
<u>U_n</u> 30V t 15μs n2	
<u>U''</u> 5V t 30s	

Example of a display of electrofusion with activated alignments U'_{\sim} / U''_{\sim} .

- Use the arrow keys to modify the parameter in the direction required. According to the parameters selected for U'_{\sim} and U''_{\sim} , fusion can be carried out without alignment or in combination with one or both alignment functions as required.

12.AUG.00	13:42
<u>U'</u> 0V t 30s	
<u>U_n</u> 30V t 15μs n2	
<u>U''</u> 5V t 30s	

Example of the display for carrying out electrofusion with inactivated alignment U'_{\sim} .

The relevant parameters appear in the display in larger letters and in bold print.

U'_{\sim} / U''_{\sim} :

Alternating voltage, in V (volt).

Can be set to "0" and in increments of 0.1 V in the range 1.0–10 V.

t: Duration of alignment 0–95 s, in s (seconds). Can be set in increments of 5 seconds.

U_n : Voltage, in V (volt). Can be set to "0" and in increments of 1 V between 5 V and 100 V, and in increments of 10 V between 100 V and 300 V.

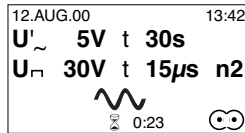
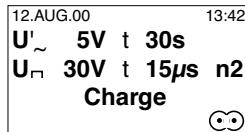
t: Duration of pulse, in μs (microseconds). Can be set in increments of 5 μs between 0 and 15 μs–300 μs.

n: Number of pulses 0–99. Can be set in one-pulse increments.

5 Mode of operation

Step 2 – Starting cell fusion


- Trigger the cell fusion process by Pressing the START key.
- "Charge" appears briefly in the fourth line of the display. Charging commences.
- During the charging procedure, alternating voltage U_{\sim} is applied for the selected duration. The alternating voltage is represented by the symbol \sim (sine-wave oscillation).
- Discharge occurs after the pre-counted alignment time (0–95 seconds). This is indicated by a flash in the fourth line of the display. If the number n which has been entered is $n > 1$, a further discharge occurs each time after one second.



5 Mode of operation

- After the discharging procedure has ended, the alternating voltage U''_{\sim} is applied for the set duration (0–95 seconds). The overall time remaining for the experiment is constantly visible on the bottom line of the display.

After the experiment has ended, a double acoustic signal is emitted and the initial menu appears in the display with the parameters which have used.

12.AUG.00	13:42
U'_{\sim} 5V t 30s	
U_{\square} 30V t 15 μ s n2	
U''_{\sim} 5V t 30s	
READY SINCE 1:36	

The time which has elapsed since the end of the experiment appears in the bottom line in minutes and seconds. After 9 minutes or after any key has been pressed, this information disappears from the display.

6 Error messages

In the event of an error, the Multiporator® emits an acoustic signal and an error message appears in the display or via the printer (optional). These error messages refer to device errors or to applicational errors. Errors 1–4 and 9 may occur in the mode for bacteria, errors 1–7 in the mode for eukaryotic cells and errors 1–8 in the mode for cell fusion.

Error no.	Error message	Cause	Solution
01	No Display	Display is not controlled.	Contact SERVICE.
02	No RTC / NVRAM	Electronic module for the internal clock is defective.	Contact SERVICE.
03	High Current	The current was too high during the discharging process (pulse).	Reduce the conductivity of the solution used. Check whether the correct cuvette type has been inserted.
04	No Cuvette	Cuvette has not been inserted.	Insert a cuvette. Restart the device. Push cuvette insert fully into the device.
05	Timeout Charge	The capacitor was unable to be charged in within the allotted period of time.	Contact SERVICE.
06	Timeout Charge	During repeated pulsing, the capacitor was unable to be charged within the period between the individual pulses.	Reduce the conductivity of the solution used. Check whether the correct cuvette type has been inserted.

6 Error messages

Error no.	Error message	Cause	Solution
07	Short τ	The capacitor was completely emptied; it is uncertain whether the τ has been executed. The limiting element was the capacitor and not the pulse regulator.	Reduce the conductivity of the solution used. Check whether the correct cuvette type has been inserted.
08	Low Resistance	The current in the cuvette is too high and the alternating voltage cannot be maintained.	Reduce the conductivity of the solution used.
09	Timeout Measuring τ	Timeout for the τ -measurement of the bacteria module.	Contact SERVICE.
Bacteria module	$\tau_a = 0.8$ ms, U_a is considerably lower than the parameter U which has been set.	– Sparks in the cuvette.	– Use a cuvette which has a greater distance between the electrodes.
		– The current during discharge (pulse) was too high.	– Reduce the conductivity of the solution.
		– Technical error.	– Contact SERVICE.

7 Maintenance and servicing

Warranties and servicing are the responsibility of the distributor.

7.1 Disinfection

Before disinfecting the Multiporator[®], disconnect the device from the main power supply.

All parts of the Multiporator[®], including the accessories and the connecting cable, must undergo wipe disinfection.

The cuvette insert can also undergo spray disinfection.

However, it is not advisable to carry out spray disinfection on the entire device, as disinfectant may enter the device.

7.2 Cleaning

Before cleaning the Multiporator[®], disconnect the plug.

Ensure that no fluids enter the Multiporator[®], as this could cause short-circuits in the electrical installation as well as corrosion.

Wipe painted parts and aluminum surfaces using a cloth and mild detergent and then wipe with a dry cloth.


Warning: Do not use any corrosive, solvent or abrasive detergents or polishes.

8 Technical data

Voltage/frequency:	100–240 V \pm 10 %, 50–60 Hz
Fuses:	T0, 1.0 A – 5 x 20 mm (2 pcs.)
Power consumption:	60 W
Excess-voltage category:	II
Protection class:	I
Degree of contamination:	2
Voltage range for the following modules:	
Eukaryotic cells:	20– 100 V, in increments of 1 V 100–1,000 V, in increments of 10 V 1,000–1,200 V, in increments of 100 V Interval between pulses: 1 minute
Cell fusion:	0, 5– 100 V, in increments of 1 V 100– 300 V, in increments of 10 V Interval between pulses: 1 second
Alternating voltage:	0, 1.0– 10 V, in increments of 0.1 V
Bacteria:	200–1,000 V, in increments of 10 V 1,000–2,500 V, in increments of 100 V No multiple pulse
Capacitor:	10 μ F, 2,500 V impulse discharge
Resistance:	600 Ω parallel 56 Ω series
Charging time:	<30 seconds
No. of pulses (n):	0–99
Time constant τ of discharge:	
Eukaryotic cells:	15–500 μ s, in increments of 5 μ s, fading e-function
Bacteria:	Nominal 5 ms, with an impedance of the sample of 3.3 k Ω Measurement and display from 0.8–6.0 ms
Duration of alignment:	0–95 s, in increments of 5 s
Duration t of pulse:	
Cell fusion:	0, 15 μ s–300 μ s, in increments of 5 μ s, square-wave pulse
RS 232 interface:	9,600 baud, 8 bits, no parity, 2 stop bits, XON/XOFF
Ambient temperature:	max. 40 °C
Relative ambient humidity:	max. 80 %

8 *Technical data*

Weight:	max. 5.5 kg (according to modification level)
Dimensions:	Width: 25.2 cm
	Depth: 34.2 cm
	Height: 12.0 cm

The device is -approved and has UL and CSA authorization (cUL: E 158089).

U.S. Pat. No. 6,008,038.

Technical specifications subject to change!

9 Ordering information

Order no.

4308 000.015	Multiporator [®] , basic version, for eukaryotic cells, 100–240 V, 50–60 Hz
4308 000.023	Multiporator [®] , eukaryotic cells, and modification level "bacteria mode"
4308 000.031	Multiporator [®] , eukaryotic cells and modification level "cell fusion"
4308 000.040	Multiporator [®] , eukaryotic cells and modification level "bacteria mode" and "cell fusion"

Accessories

Buffer

4308 070.501	Hypoosmolar buffer for electroporation (PH), 100 ml
4308 070.510	Isoosmolar buffer for electroporation (PI), 100 ml
4308 070.528	Hypoosmolar buffer for cell fusion (FH), 100 ml
4308 070.536	Isoosmolar buffer for cell fusion (FI), 100 ml

Electroporation cuvettes

4307 000.569	1 mm gap width, aluminum, sterile, 50 pcs.
4307 000.593	2 mm gap width, aluminum, sterile, 50 pcs.
4307 000.623	4 mm gap width, aluminum, sterile, 50 pcs.
4308 078.006	Cuvette stand for 16 electroporation cuvettes
4308 025.018	Insert for electroporation cuvettes
4308 021.004	Insert (electroporation / electrofusion) for connecting external electrodes
4308 012.005	Insert for Helix fusion chambers
4308 014.008	Helix fusion chamber (Electrode gap width: 0.2 mm)
4308 030.003	Micro fusion chamber (Electrode gap width: 0.2 mm)
4308 031.000	Micro fusion chamber (Electrode gap width: 0.5 mm)
4308 024.003	Insert for Micro fusion chamber
4308 013.001	Replacement cup for Helix fusion chamber
4308 017.007	Stand for 10 Helix fusion chambers
4308 010.002	Conversion kit for mode for bacteria (to be installed by SERVICE)
4308 011.009	Conversion kit for mode for cell fusion (to be installed by SERVICE)

9a Ordering information for North America

Order no.

940000505	Multiporator [®] , basic version, for eukaryotic cells, 100–240 V, 50–60 Hz
940000602	Multiporator [®] , eukaryotic cells, and modification level "bacteria mode"
940000700	Multiporator [®] , eukaryotic cells and modification level "cell fusion"
940000807	Multiporator [®] , eukaryotic cells and modification level "bacteria mode" and "cell fusion"

Accessories

Buffer

940002001	Hypoosmolar buffer for electroporation (PH), 100 ml
940002109	Isoosmolar buffer for electroporation (PI), 100 ml
940002150	Hypoosmolar buffer for cell fusion (FH), 100 ml
940002206	Isoosmolar buffer for cell fusion (FI), 100 ml

Electroporation cuvettes

940001005	1 mm gap width, aluminum, sterile, 50 pcs.
940001013	2 mm gap width, aluminum, sterile, 50 pcs.
940001021	4 mm gap width, aluminum, sterile, 50 pcs.
940001102	Cuvette stand for 16 electroporation cuvettes
940004225	Insert for electroporation cuvettes
940004209	Insert (electroporation / electrofusion) for connecting external electrodes
940004268	Insert for Helix fusion chambers
940001200	Helix fusion chamber (Electrode gap width: 0.2 mm)
940001251	Micro fusion chamber (Electrode gap width: 0.2 mm)
940001234	Micro fusion chamber (Electrode gap width: 0.5 mm)
940004241	Insert for Micro fusion chamber
940004187	Replacement cup for Helix fusion chamber
940001218	Stand for 10 Helix fusion chambers
940004101	Conversion kit for mode for bacteria (to be installed by SERVICE)
940004128	Conversion kit for mode for cell fusion (to be installed by SERVICE)

EG-Konformitätserklärung EC Conformity Declaration

Das bezeichnete Produkt entspricht den einschlägigen grundlegenden Anforderungen der aufgeführten EG-Richtlinien und Normen. Bei einer nicht mit uns abgestimmten Änderung des Produktes oder einer nicht bestimmungsgemäßen Anwendung verliert diese Erklärung ihre Gültigkeit.

The product named below fulfills the relevant fundamental requirements of the EC directives and standards listed. In the case of unauthorized modifications to the product or an unintended use this declaration becomes invalid.

Produktbezeichnung, Product name:

Multiporator® 4308

Produkttyp, Product type:

Elektroporator / electroporator

Einschlägige EG-Richtlinien/Normen, Relevant EC directives/standards:

2006/95/EG, EN 61010-1

2004/108/EG, EN 55011/B, EN 61000-6-1, EN 61000-3-2, EN 61000-3-3



Vorstand/Board of Management:

21.05.2007

Hamburg, Date:

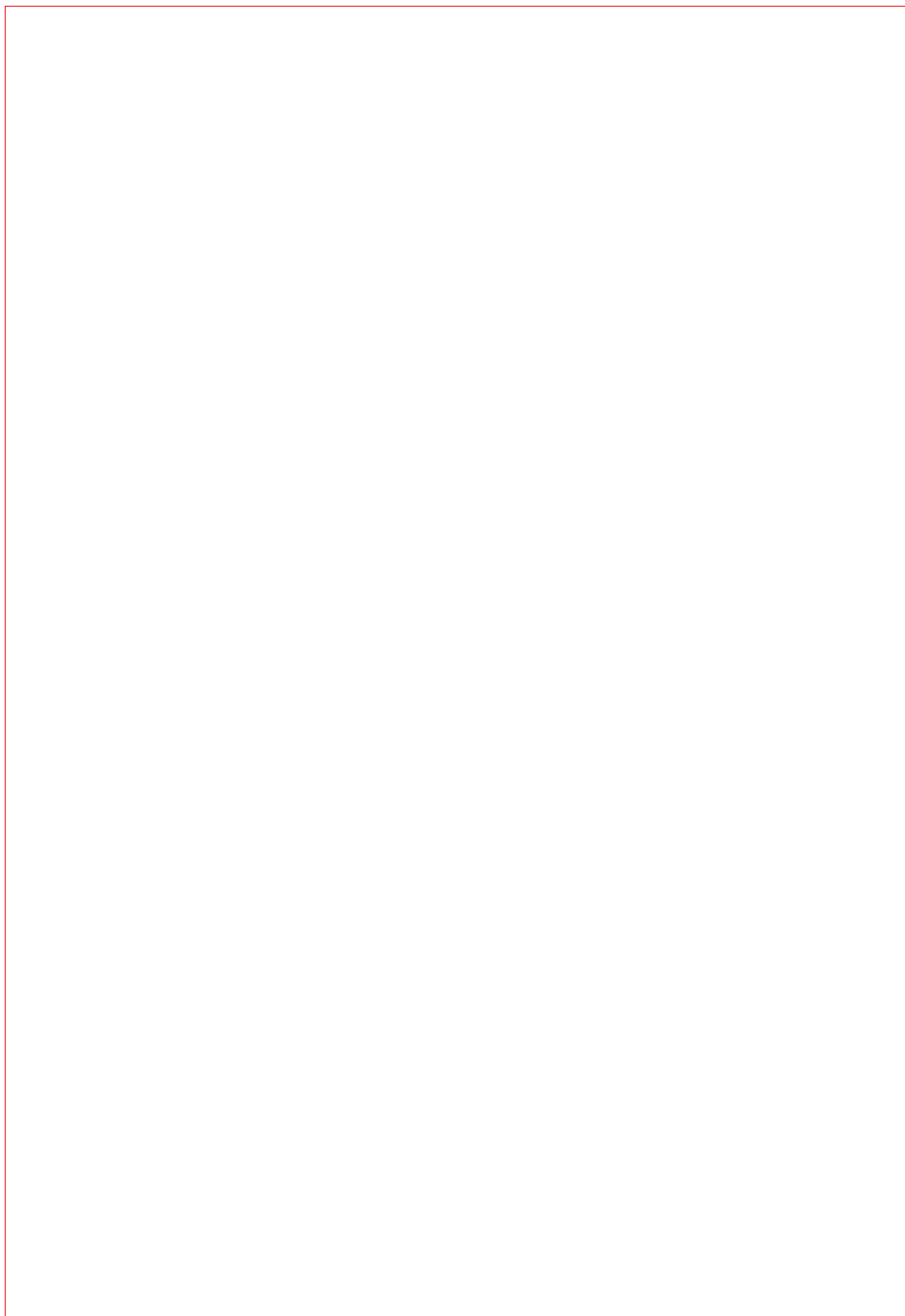


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