FemtoJet®

Operating Manual

eppendorf

Contents

Contents

1 Safety precautions and application

- Observe operating instructions!

- Before plugging in the device, please compare your voltage requirements with the specifications on the identification plate!

- The unit must be connected to an earthed socket!

- Electrical connections may be effected only when the device is switched off!

- The unit may be opened only by authorized service personnel! Potentially lethal voltage inside the unit.

- Do not use the unit if damaged. This applies in particular when the mains cable is damaged.

- Electrical connection with devices not expressly mentioned in this operating manual is permitted only after obtaining prior approval from the manufacturer!

- If the unit is not used in accordance with purpose for which it is intended, its protective function may be impaired.

- ⚠ Caution!
  Take care when mounting glass capillaries or tools! Mounting must always take place without the use of pressure! Capillaries which have been mounted incorrectly may break loose under pressure. Never point mounted capillaries at persons.

- ⚠ Caution!
  Take care when using glass capillaries! During mounting or when placed upon a hard surface, the glass capillaries may break and glass splinters may cause injury. It is therefore essential to wear eye protection!

1 Safety precautions and application

- In the case of independently-manufactured capillaries, ensure that the outer diameter of the capillary corresponds to the specifications of the capillary grip head.

- In the case of extended periods of non-use, the unit should be depressurized using the `Standby` key and switched off before being stored!

- Use the unit only for the described purpose! The unit and in particular the high pressure may not be used for purposes other than microinjection!

- Use only original Eppendorf accessories. Only original accessory parts may be used. The only exceptions are auxiliary pieces of equipment recommended by the manufacturer. Otherwise all claims under guarantee and liability become void immediately. Use other accessories only if you have a certificate from Eppendorf AG confirming technical safety in use.

- The responsibility for the correct functioning of the device passes onto the owner or user if the device is maintained or serviced improperly by persons not belonging to the Eppendorf service team or if used for a purpose other than that intended. Eppendorf AG is not liable for damage occurring from non-observance of these notes. Guarantee and conditions of liability of the terms of sale and delivery of Eppendorf AG are not expanded by the above notes.

- The Eppendorf AG reserves the right to make technical alterations to this device!

2 Device description

The FemtoJet® enables minute quantities of liquid to be injected into cells. The relevant parameters (injection pressure p_i and injection time t_i) are set using the variable regulators. The $\boxed{\text{Inject}}$ key or connected hand or foot control or the INJECT key of the connected micromanipulator are used to trigger these two parameters. If no injections are being performed, compensation pressure p_c is applied permanently.

2 Device description

2.1 Overview
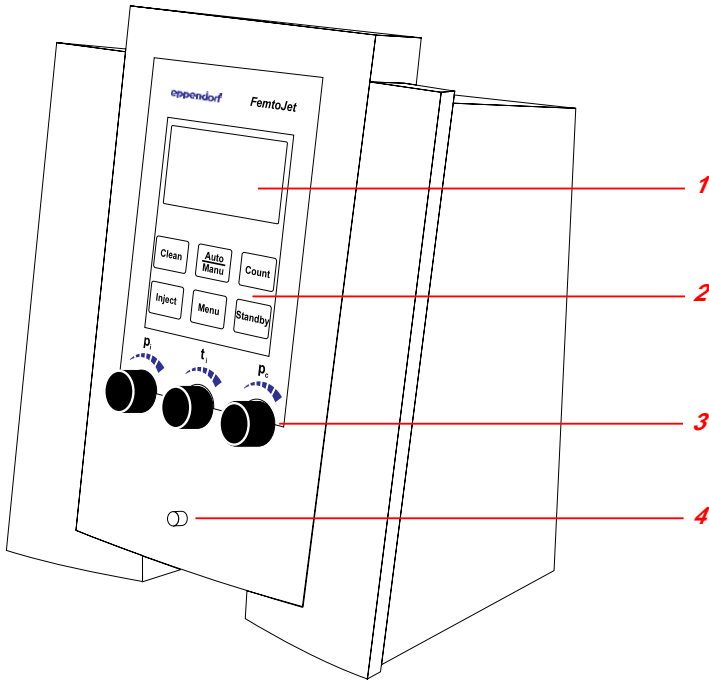
1 **Display**
– Displays relevant parameters during injection or setting
of parameters.
– Guides operator through functions.

2 **Keypad**
– To trigger actions.
– To call up the function menu.
– To switch to and from Standby mode.

3 **Variable regulators**
– To set parameters injection pressure p_i, injection time t_i and
compensation pressure p_c.
– To select functions and parameters in Function mode.

4 **Tube connection**
– To connect the pressure tube to the device via the bayonet joint.

2 Device description

Standard accessories:

1 Pressure tube, 2 m
1 Universal capillary holder with grip head for capillary diameters
  of 1.0 mm and adapter for Femtotips®
1 Connecting cable (Interface Cable 5171) to the Micromanipulator 5171
  or InjectMan®
1 Hand control for triggering injection and the Clean function
1 Femtotips®, Femtotips® II, Microloader and CELLocate demo pack
1 Mains cable
1 Operating manual

Optional accessories:

1 Foot control for triggering the Inject function

2 Device description

2.2 Short description

Display

Display of all relevant parameters and information.

Function keys

| Clean | Auto / Manu | Count |
| Inject | Menu | Standby |
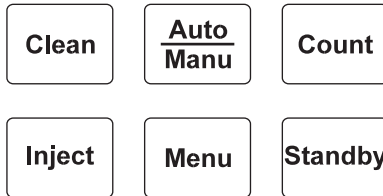
Clean

| Clean |

Clean
To apply the maximum pressure for cleaning the capillary to the tube outlet.

Auto / Manu

| Auto / Manu |

Auto / Manu
To switch between manual and automatic time-controlled injection.

Count

| Count |

Count
To reset to zero the counter for injections which have been actuated.

Inject

| Inject |

Inject
To execute automatic or manual injection.

Menu

| Menu |

Menu
To switch the device to menu control; selected functions may be executed.

Standby

| Standby |

Standby
When the key is pressed briefly:
To switch the device between operating mode and standby mode.
When the key is held down:
As above, but also deairs the pressure accumulator.

2 Device description

Variable regulator
In the normal operating mode, the parameters for injection are set by the three variable regulators. The arrangement of the variable regulators corresponds to the arrangement of the parameters in the two bottom lines of the display:
From left to right: injection pressure pi, injection time ti and compensation pressure p_c.
In other operating modes, the variable regulators have either the significance indicated in the display or no significance at all.

Tube connection
The pressure tube is connected to the device via a bayonet joint.

2 Device description

2.3 Startup
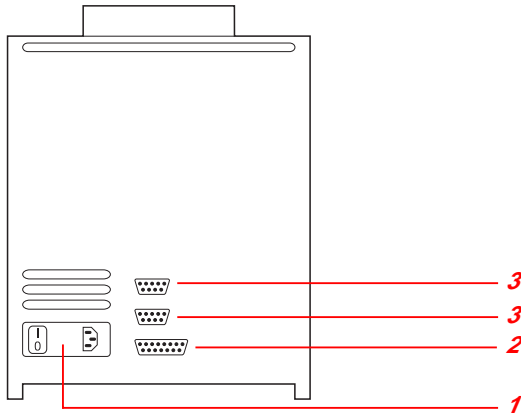
Diagram of rear of device

- – Electrical connection is effected via the mains socket with integrated mains switch (1).

- – Connection to an Eppendorf manipulator designed for injection is effected via the 15-pin interface (2).

- – Optional switches (e.g. foot control, hand control) or a computer can be connected to the 9-pin interfaces.

Switching on the device
Set the mains switch on the rear of the device to "I". The unit is switched on, the display lights up, the unit runs through a warm-up routine and is then ready for operation. The injection mode is shown in the display.

After being switched on, the unit runs through an initialization phase and supply pressure is built up. This takes approximately three minutes.

2 Device description

Switching off the device

If it is not going to be used for an extended period, the device should be depressurized via the switch-off routine using the `Standby` key. This guarantees a long service life of the device (see Section 3.1.3).

The switch-off routine ensures that the system is dewatered and deaired correctly. Do not disconnect the device from the mains supply until dewatering and deairing have taken place. If only the mains switch is actuated, the existing pressure remains in the system.

3 Mode of operation

3.1 Display, variable regulators and keypad

Before operating the device for the first time, users should read this section carefully. This section describes operation of the device only. Handling of the injection capillaries is described in Section 3.3.

```
AUTO                n= 0

  pi=  150  hPa

pi[hPa]    ti[s]    pc[hPa]
  150       0.2        50
```

```
AUTO                n= 0

  pi=  215  PSI

pi[PSI]    ti[s]    pc[PSI]
  2.15      0.2       0.72
```

Display
The display shows the following:

1st line:
injection mode AUTO or MANU, counter n for number of injections performed

2nd line (bold):
The most-recently set parameter or relevant parameter

Bottom lines:
Parameters which affect injection

Compensation pressure p_c which is applied permanently in the normal mode is displayed as an actual measured value.

The arrangement of the three parameters shown, p_i, t_i, p_c, corresponds to the arrangement of the variable regulators.

3.1.1 Setting the injection parameters

The injection parameters have to be adapted to the injection capillaries used and the cell type and liquid which are to be injected.

The following injection parameters can be set using the variable regulators:

Injection pressure	p_i	0 – 6,000 hPa (0.00 – 87.0 psi)
Injection time	t_i	0.1 – 99.9 s
Compensation pressure	p_c	0 – 6,000 hPa (0,00 – 87.0 psi)

The settings are in increments of 1 hPa/0.1 s. The display can show hPa or psi, as required. The setting and internal pressure conversion are always performed in hPa. Rounding for the display in psi can result in increments of 0.01 to 0.02 psi.
The display of pressure in psi varies according to the size of the value (e.g.: 9.99 may also appear in the display as 10.0).

3 Mode of operation

Slight deviations between the nominal value (specified value) and displayed compensation pressure p_c (actual value) may occur for technical reasons. The relevant value is the actual value measured.

The following settings are used as a basis for initial injection experiments using Femtotip® / Femtotip® II:

Display:

```
AUTO                    n= 0

     pc=  50  hPa

pi[hPa]    ti[s]    pc[hPa]
  150      0.5        50
```


p_i left-hand variable regulator: 150 hPa
t_i central variable regulator: 0.5 s
p_c right-hand variable regulator: 50 hPa

Variable regulators:
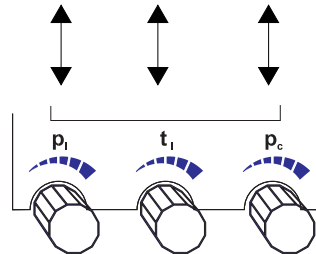
3.1.2 Variable regulators

The variable regulators are set so that when they are turned slowly, the values are changed by 1 hPa / 0.1 second per notch (0.01 – 0.02 psi). If the regulators are turned faster, the values are altered in larger increments.

3 Mode of operation

3.1.3 Keypad

Inject **Inject**

Performs automatic or manual injection. Automatic injection is controlled via the injection time t_i, and the injection pressure p_i is used. In the second line, injection time t_i is counted down from the nominal value.

```
AUTO - INJECT          n= 0

          0.4  s

pi[hPa]      ti[s]      pc[hPa]
  150        0.6          50
```

Manual injection is performed for as long as the Inject key is held down. Injection pressure pi is used. In the second line, injection time ti is counted upwards from 0.0.

If the FemtoJet® is connected to the Eppendorf Micromanipulator 5171 or Inject Man®, the injection movement of the micromanipulator is also started via the Inject key. Both units then combine as a system to perform semi-automatic microinjection.

Alternatively, the INJECT key of the connected micromanipulator or the hand/foot control can be used.

If synchronization is set to "limit" (SYNC = LIMIT) in the INJECT function of the micromanipulator, semi-automatic injection can only be triggered via the micromanipulator.

Clean **Clean**

The maximum available pressure for cleaning is applied at the tube outlet for as long as the key is held down. Once the key is released, the pressure drops to compensation pressure p_c again. The pressure curve is shown in the form of a bar.

```
CLEAN                  n= 0

═══════════════_____

pi[hPa]      ti[s]      pc[hPa]
  150        0.2          50
```

3 Mode of operation

Auto / Manu

<kbd>Auto Manu</kbd>

To switch between manual and automatic, time-controlled injection.

In the case of manual injection, time is counted upwards from 0.0 seconds. The parameter t_i is not displayed in the bottom two lines in order to emphasize that the time cannot be set via the variable regulators in this case.

```
MANU - INJECT        n= 0

           0.4 s

pi[hPa]              pc[hPa]
  150                  50
```

Count

<kbd>Count</kbd>

To reset to zero the counter for injections which have been actuated.

Menu

<kbd>Menu</kbd>

To switch the devict to menu control; selected functions may be executed.

In the first line, FUNC indicates that the device is in the function menu and shows which function number is active. The second line contains the function description (e.g. "Select pressure unit").

```
FUNC  1
Select pressure unit
INJECT = PSI         [hPa]

Turn pi to
select function
```

The third line requests an action (e.g. press the <kbd>Inject</kbd> key to change the pressure unit). Current status: [hPa]

The two bottom lines show information about which action is assigned to which variable regulator (e.g. other functions can be selected using left-hand variable regulator p_i).

3 Mode of operation

Standby
in the case of a brief interruption

When key is pressed briefly:
The device switches to the Standby
mode **without** deairing. The illumination
of the display switches off. "STANDBY"
appears in the display with the suffix
"Supply pressure = rxyz" as a code for
system pressure p_s being maintained.

Application:
For temporary interruptions or use of
Standby as a Reset function.

When key is pressed again:
The device is reactivated and runs
through a short warm-up routine.
This lasts approximately one minute.

STANDBY

Supply pressure   6789
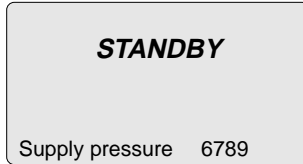
Standby **Standby**
with deairing of pressure accumulator

Hold down the key (approx. 2 seconds;
the display moves to "STANDBY"):

The device switches to the Standby
mode. The pressure accumulator is
completely deaired, the illumination of
the display switches off. "STANDBY"
appears in the display with the note
"Supply pressure = x" (x ~ 0).

A noise can be heard at this stage.

Application:
For correct shutdown, with deairing of the
entire pressure system in the event of
longer periods of non-use or standstill
(weekend or storage) with subsequent
switch-off using the mains switch.

Press the key again:
The device is reactivated and runs
through the warm-up routine.
This takes approximately three minutes.

Hold standby
to exhaust

STANDBY

Supply pressure   0
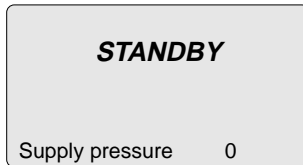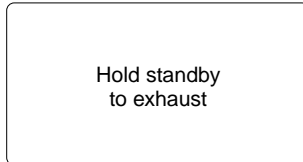
3 Mode of operation

Reset

To reset the device to the initial mode by pressing the Standby key briefly or via the mains switch

The pressure accumulator is not deaired.

3.2 Hand control / foot control
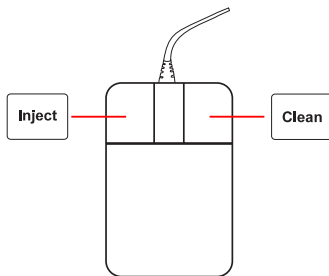
The functions of the hand/foot control correspond to the functions of the Inject and Clean keys on the FemtoJet®.

The centre key of the hand control has no function.
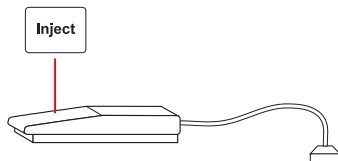
The function of the foot control corresponds to the Inject key.

3 Mode of operation

3.3 Connecting the capillary holder

Universal capillary holder

The universal capillary holder is used to locate the injection capillaries. Prefabricated Eppendorf Femtotips® / Femtotips® II (6) are screwed into the universal capillary holder using the adapter (5). To do so, the adapter for Femtotips® (5) is first screwed into the front rotatable knurled screw (4) and then the Femtotip® / Femtotip® II is attached. If self-pulled capillaries are to be attached, the grip head 0 (7) needs to be screwed loosely into the front rotatable knurled screw (4). The self-pulled capillary is pushed into the front opening of the grip head through the two O-rings and fixed in position by tightening the grip head.
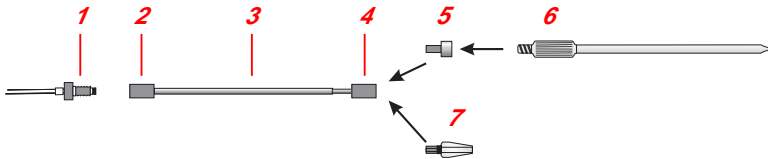
Universal capillary holder

1   Tube connection
2   Rear knurled screw
3   Tension piece
4   Front knurled screw, rotatable
5   Adapter for Femtotips® (microcapillaries for microinjection)
6   Femtotip® / Femtotip® II
7   Grip head 0, plastic, with two O-rings and sealing washer

Connecting the universal capillary holder

The two-meter-long pressure tube is connected to FemtoJet® (1) via the bayonet joint (2). The pressure tube (3) is screwed to the rear knurled screw (4) of the universal capillary holder. Femtotips® / Femtotips® II are fitted at the front end of the universal capillary holder (5) or independently-manufactured capillaries are clamped with the aid of the appropriate grip head.
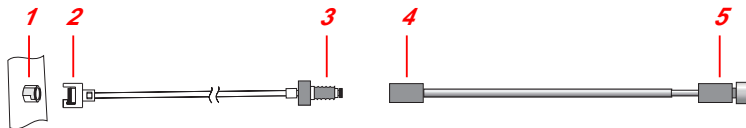
3 Mode of operation

Other accessories
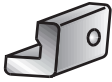
Clamping piece
for fastening the universal capillary holder.
Not required for TransferMan® NK or for Eppendorf
micromanipulators, which have an adjustable
cannula holder.
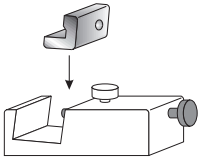
The universal capillary holder is mounted to the
micromanipulator by placing the clamping piece into
the tool holder of the Eppendorf Micromanipulator
and then tightening it with the fastening screw.

The universal capillary holder is positioned with
the correct length into the tool holder and then fixed
into position. The position of the capillary can be
changed by loosening the fastening screw.
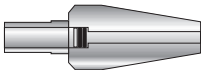
Grip head 0,
incl. 2 sets of O-rings and sealing washer.

Using grip head 0 supplied, capillaries with an outer
diameter of 1.0 mm to 1.1 mm can be used. In the
case of outer diameters which differ from these
measurements, the relevant grip heads can be
ordered as non-standard accessories for the
universal capillary holder (see Section 7, Ordering
information). The transparent grip head allows the
user to visually monitor the position of the capillary.

3 Mode of operation

3.4 Information on working practices

The following pressures can be used:

Name	Pressure range	Comments
Compensation pressure p_c	0 – 6,000 hPa 0.00 – 87.0 psi	
Injection pressure p_i	0 – 6,000 hPa 0.00 – 87.0 psi	
Clean pressure	maximum up to 7,000 hPa maximum up to 102 psi	
Constant working pressure p_w	0 – 6,000 hPa 0.00 – 87.0 psi	Select Function 4 via the ⟨Menu⟩ key.
System pressure p_s [supply pressure]	maximum up to 7,000 hPa maximum up to 102 psi	This is used directly for ⟨Clean⟩. In certain phases of initialization, the system pressure may temporarily increase to a maximum of 8,000 hPa (117 psi).

3 Mode of operation

3.4.1 Compensation pressure p_c

The compensation pressure p_c ensures that no medium flows into the capillary. Capillary forces would make liquid flow out of the cell culture dish into the injection capillary and thus dilute the injection material. To prevent this, a permanent compensation pressure p_c is set. This should be selected so that there is a permanent slight flow-out of liquid from the injection capillary. The individual pressure level can be determined in a preliminary test. For example, a fluorescent dye can be added to the capillary to enable outflow to be monitored directly. The pressure required is heavily dependent on the surface tension and viscosity of the injection material. Experience has shown that compensation pressures of between 30 hPa and 300 hPa (0.44 – 4.4 psi) are appropriate values for ready-to-use Eppendorf Femtotips® injection capillaries.

Capillary suction

Compensation pressure p_c

Hydrostatic pressure P hyd
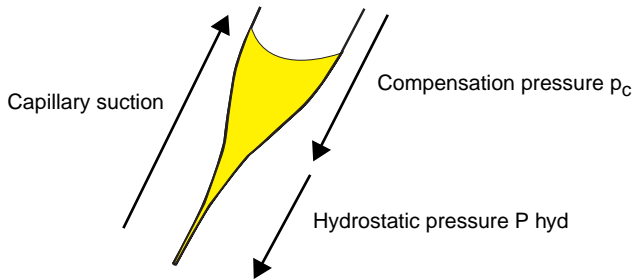
Pressure in the capillaries

3.4.2 Injection pressure p_i

Injection pressure p_i is applied during the injection process. This pressure is generally higher than the compensation pressure. In many cases, a pressure between 50 hPa and 500 hPa (0.73 – 7.2 psi) is appropriate for Femtotips®. The volume injected is dependent on the injection pressure and the injection time. The injection pressure must be greater than the internal pressure of the cell into which injection should take place. However, if the cell or the cell nucleus swell up during injection, the injection pressure is too high.

If the compressor starts up occasionally during experiments, this does not impair the function of the FemtoJet®. Pressure is always available up to the maximum value of 6,000 hPa (approx. 87.0 psi), even when the compressor is topping up the supply pressure.

3.4.3 Injection time t_i

Injection time t_i gives the period for which injection pressure p_i is maintained. t_i is usually selected from a range between approx. 0.3 s and 1.5 s. The relevant factor for the injected volume is the product of the injection pressure and the injection time.

Depending on the setting on the Eppendorf Micromanipulator which is connected, the injection time t_i is measured from the point at which the | Inject | key is pressed. The injection pressure is built up immediately, so it is already present during the injection movement and during penetration of the cell. Once the end of injection time has been reached, the device switches to compensation pressure p_c. At the same time, the micromanipulator is moved back into the working level. When "Injection at Z limit" is set, measurement of the injection time t_i does not begin until the Z limit has been reached. Once t_i has elapsed, the FemtoJet$^®$ switches back to p_c and the micromanipulator moves back into its starting position in the working level.

3.4.4 Clean function

The Clean function is used to flush out blocked capillaries. The maximum available pressure is applied to the outlet with the | Clean | key. It is applied for as long as the | Clean | key is pressed. The maximum pressure of the FemtoJet$^®$ is in the range of 7,000 hPa (102 psi). The value cannot be changed.

The pressure curve up to maximum pressure appears in the display in the form of a bar chart. By pressing the | Clean | key briefly, a lower pressure can also be obtained.

3 Mode of operation

3.4.5 Manual or automatic injection

In the Manu operating mode, injection is performed for as long as the $\boxed{\text{Inject}}$ key or hand / foot control is held down. The duration (t_i) of injection is measured and shown in the display. In the case of combination with an Eppendorf Micromanipulator, the injection movement is triggered.

In the Auto operating mode, the built-in timer facilitates a reproducible injection time. The injection runs automatically for the duration of set time t_i. It is triggered by the $\boxed{\text{Inject}}$ key or the hand / foot control being pressed briefly. During injection, the Inject key is inactive, i.e. pressing it again has no effect. In combination with an Eppendorf Micromanipulator 5171 / InjectMan®, the automatic injection procedure can also be triggered from the operating panel of the micromanipulator.

3.4.6 Constant pressure/constant working pressure

Using the Constant Pressure function, it is possible to select a higher pressure permanently if required. This function is helpful when special injection techniques require either a high pressure up to a maximum of 6,000 hPa (87 psi) or a permanent flow of injection solution (see Section 3.7.5). Control accuracy drops in the pressure range from 2,000 – 6,000 hPa (29 – 87 psi).

3.4.7 General note

FemtoJet® is fitted with an integrated compressor. For this reason, the compressor may start up or excess pressure may be let off briefly before, during and after injections or during periods when the FemtoJet® is not being used. This is not a malfunction of the device and does not impair injection.

In the case of injections under high pressure at very short intervals or in the case of the Clean function being carried out several times in succession, the pressure in the system accumulator may drop significantly. This is indicated by a warning or an error message. In this case, wait a short time until the supply pressure has stabilized again. It is then possible to continue working immediately.

3 Mode of operation

3.5 The first injection

The following are available:

- Adherent cells in a Petri dish (possibly on CELLocates to observe specific cells after injection),
- Injection liquid (purified, e.g. by centrifugation),
- Femtotips® / Femtotips® II; other capillaries as an alternative
- Microloader,
- 0.5 – 10 µl pipette to take the Microloader,
- A universal capillary holder on the micromanipulator which has been preadjusted with a Femtotip®.

Filling the Femtotips® with the Microloader:

- Select function 0 "Capillary exchange" by pressing $\boxed{\text{Menu}}$. Connect the pressure tube.
- Aspirate approximately 0.5 – 5.0 µl of centrifuged injection fluid with the Microloader without touching the base of the vessel. Using the Microloader, fill the Femtotip® through the rear opening, inserting the tip approximately up to the centre of the Femtotips® and releasing the fluid there.
- Hold the Femtotip® vertically, loosen the cap by turning it and allow it to drop vertically.
- Screw the Femtotip® into the capillary holder.
- Complete Function 0 by pressing $\boxed{\text{Menu}}$. Compensation pressure is applied.
- Move the capillary into the cell medium (Petri dish) with the micromanipulator.
- Focus the cells with the microscope, position the capillary slightly above the cells but do not allow it to come into contact with the base of the Petri dish. Focus the capillary.
- Using the $\boxed{\text{Clean}}$ key, check that the capillary is not blocked. Either the fluid can be seen flowing out or it is rendered visible by the fluorescent substance it entrains. As a rule, any air bubbles present are blown out.
- Perform a sample injection using the preset parameters (e.g. $p_i \sim 150$ hPa, $t_i \sim 0.5$ s, $p_c \sim 50$ hPa, corresponding to $p_i \sim 2.18$ psi, $t_i \sim 0.5$ s, $p_c \sim 0.73$ psi). Injection can be monitored visually when the cell size changes by approx. 5 % to 10 % or via fluorescence. If required, adapt parameters p_i and/or t_i.

3 Mode of operation

3.6 Connecting an Eppendorf Micromanipulator

Connection to an Eppendorf Micromanipulator designed for automated injection is effected with the 15-pin interface.
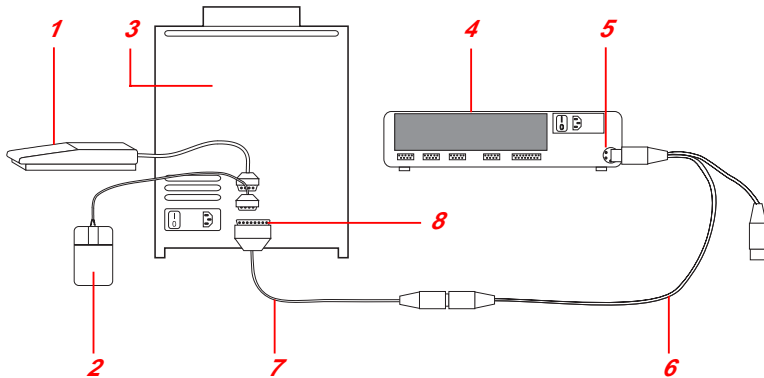
FemtoJet$^{®}$ (rear) with all connection options

1   Foot control (optional) with Inject function
2   Hand control with Inject and Clean functions
3   FemtoJet$^{®}$
4   Micromanipulator 5171 or InjectMan$^{®}$
5   Connection socket of micromanipulator for
    Transmission cable 5246 ("Y cable")
6   Transmission cable 5246
    (accessory for Micromanipulator 5171 and InjectMan$^{®}$)
7   Interface cable 5171
    (Connection from FemtoJet$^{®}$ to Transmission cable 5246 "Y cable")
8   Interface of FemtoJet$^{®}$ to micromanipulator

3 Mode of operation

3.7 Functions

The Function menu is called up using the [Menu] key. One of seven functions is selected using left-hand variable regulator p_i. The relevant functionality is assigned to the variable regulators in the bottom line of the display.

Exit the Function menu by pressing the [Menu] key again.

In cases of error, pressure control is switched off from within the Function menu. Then exit the Menu using the [Menu] key.

Function 6, self-test, is described in Section 5, "Troubleshooting".

3.7.1 Changing the capillaries

```
FUNC  0
Change capillary
        Capillary may be
        changed now
Turn pi to
select function
```

FUNC 0
When this function is called up, the pressure tube is deaired and any pressure present in the system is let out. Once the capillary has been changed, exit the function using the [Menu] key or variable regulator p_i; pressure control will restart.

3.7.2 Setting the pressure unit

```
FUNC  1
Push Inject to select
Pressure Unit      hPa   PSI

Turn pi to
select function
```

FUNC 1
The other pressure unit is selected using the [Inject] key and all pressure values are converted. The pressure unit currently selected (in this case: hPa) is underlined.

3 Mode of operation

3.7.3 Switching on/off the loudspeaker

```
FUNC 2
Push Inject to select
Beeper          ON  OFF

Turn pi to
select function
```

FUNC 2
Use the | Inject | key to switch the loudspeaker on or off. The currently selected setting (in this case: ON) is underlined. Acoustic error messages are not emitted if the setting is OFF.

3.7.4 Switching on/off the background illumination of the display

```
FUNC 3
Push Inject to select
Illumination    ON  OFF

Turn pi to
select function
```

FUNC 3
Use the | Inject | key to switch the illumination on or off. The currently selected setting (in this case: ON) is underlined.

In the normal operating mode, illumination switches off after three minutes if no operating control has been actuated. When an operating control is actuated, the illumination lights up again.

3.7.5 Constant pressure

```
FUNC 4
Continuous flow
INJECT = start

Turn pi to
select function
```

FUNC 4
This function can be used to apply a constant working pressure pw in the range from approx. 0 to 6,000 hPa (87.0 psi) at the pressure outlet. The pressure unit psi can also be used.

```
FUNC 4
Continuous flow
  pw= 50 hPa

Turn pi to        pw[hPa]
select function      50
```

The working pressure p_w is set with the right-hand variable regulator (p_c), the specified value is shown in large characters. The actual value is shown in the bottom right of the display.

The Clean function can be activated. The pressure of the Clean function is shown in the bottom right of the display in the form of p_w.

3 Mode of operation

3.7.6 Dewatering the pressure accumulator

FUNC 5
Drain pressure supply
INJECT = start

Turn pi to
select function

FUNC 5
– Briefly opens the dewatering valve
of the pressure accumulator; any
condensed humidity present is blown
away.
– When this function is performed, the
noise of air being blown away can be
heard.

This function is automatically performed
every time the unit is switched on. It can
be performed manually as described if
condensation is suspected to be present.

4 Care and maintenance

4.1 Cleaning

Before cleaning the FemtoJet®, disconnect the plug.

Ensure that no fluids enter into the inside of the injector in order to avoid short-circuits in the electrical installation as well as corrosion.

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

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

4.2 Disinfection

Clean the FemtoJet® before disinfecting.

Wipe every part of the FemtoJet®, including accessories and connector cables, with disinfectant.

Spray disinfection is not advisable, as disinfectants can enter into the microinjector.

The disinfection method used must comply with current disinfection regulations and guidelines.

4.3 Maintenance

Regular maintenance is not required.
The dealer is responsible for warranty and service.

5 Troubleshooting

5.1 Potential errors

– Pressure loss due to capillary not fitted with capillary holder
connected.
Solution: Connect the capillary or select Function 0, Change capillary,
via the | Menu | key and variable regulator p_i.

– Capillary is blocked.
Solution: Press the | Clean | key for long enough.
Change capillary.
Purify injection fluid (e.g. by means of centrifugation).

– Compressor runs for a long time.
Solution: Take a short break.
Do not use the | Clean | key too often within a short period.
If possible, set a lower compensation pressure p_c.
Perform self-test (Section 5.1.1).

– Valve rattles
Solution: Connect tube to filled capillary. Pressure control is designed
for the tube connected.

– No communication with the micromanipulator.
Solution: Check cables and connections.

5.1.1 Self-test

FUNC 6
Self-Test
INJECT = Complete
CLEAN = Leak only
Connect closed tube !

Turn pi to
select function

FUNC 6
The FemtoJet® is checked for leaks in
the self-test.

This self-test can also be used if a leak is
suspected.

The test runs automatically.

The pressure tube must be connected to
a closed capillary (e.g. melted closed or
filled) for the pressure control to work
properly.

```
FUNC 6
Self-Test
Compressor          _____ s
Ps leak      _____  _____ hPa
Pw leak      _____  _____ hPa
Injection    _____  _____ hPa

ps=7000    pw=     10 hPa
```

Inject key: Complete self-test

– Checks compressor run time up to the point when a specific pressure is reached and thus for leaks in the area of the compressor.

– In the case of two different pressures, checks for leaks in the pressure accumulator (p_s) and for the internal tightness of the valves.

– Checks for leaks in the area of working pressure (p_w) in the case of two different pressures.

– Checks behavior during injection at different pressures (short continuous test).

If certain values are outside the specified range, an error message is emitted. If this recurs, inform Service.

```
FUNC 6
Self-Test

Ps leak      _____  _____ hPa
Pw leak      _____  _____ hPa

ps=7000    pw=     10 hPa
```

Clean key: Self-test to search for leaks

– Checks for leaks in the pressure accumulator (p_s) in the case of two different pressures, as well as for inner tightness of the valves.

– Checks for leaks in the area of working pressure (p_w) in the case of two different pressures.

If certain values are outside the specified range, an error message is emitted. If this recurs, inform Service.

5 Troubleshooting

5.2 Warnings

As a rule, warnings are emitted if certain operating conditions are not (yet) reached or if an action is not possible.

Acknowledge the warning with the $\boxed{\text{Inject}}$ key and the unit will return to normal status.

```
WARNING 001

Please wait for
supply pressure

INJECT = Continue
```

Warning 1
Supply pressure has not yet been reached.

The minimum supply pressure required for an injection or the Clean function was not present when the function was triggered.

Possible cause:
Multiple triggering of Clean or very rapid injection at a high injection pressure.

Solution:
Wait until the supply pressure has built up and trigger again.

```
WARNING 002

Check capillary

e.g. broken/missing ?

INJECT = Continue
```

Warning 2
Compensation pressure cannot be maintained.

Possible cause:
Capillary or capillary holder not attached or only connected loosely.

Solution:
Check all connections and tighten. Activate Function 0 to change the capillary and when the capillary holder is open.

5 Troubleshooting

WARNING 003

Switch on manipulator
or disconnect cable

INJECT = Continue

Warning 3
Micromanipulator does not react

A micromanipulator is connected but
does not react to a start pulse from the
FemtoJet®.

Possible cause:
Micromanipulator is switched off.
INJECT function on manipulator is not
released.
No limit is set on the micromanipulator.
In the INJECT function on the micro-
manipulator, synchronization is set to
limit (SYNC = LIMIT).

Solution:
Switch on the micromanipulator and
make it operational.
Disconnect the cable to micro-
manipulator.
With SYNC = LIMIT, trigger semi-auto-
matic injection via the micromanipulator
only.

5.3 Error messages

Error messages are emitted if certain operating conditions are not
reached or the device records a deviation from specified conditions.

Acknowledge the error message with the [Inject] key and the unit will
return to normal status. The measures described under "Solution" should
be carried out. If the error message recurs after acknowledgement with
the [Inject] key, inform Service.

5 Troubleshooting

```
        ERROR 010

      Supply pressure
     cannot be reached

   INJECT = Continue
```

Error 10
Supply pressure cannot be reached

The compressor runs without the maximum supply pressure being reached.

Possible cause:
High compensation pressure when the capillary holder is not attached or is poorly attached.

Technical cause:
Compressor does not start up. Leak in compressor or pressure accumulator.

Solution:
Check the capillary holder and tube for leaks or tighten up the tube connections. Service.

```
        ERROR 011

      Supply pressure is
          too high

   INJECT = Continue
```

Error 11
Supply pressure is too high

Supply pressure has exceeded the maximum permissible supply pressure.

Possible cause:
supply pressure is not relieved by the relevant standby function.
Technical defect.

Solution:
Switch off using the `Standby` key (hold down for approximately two seconds). Service.

```
        ERROR 012

      Supply pressure is
        does not fall

   INJECT = Continue
```

Error 12
Supply pressure does not drop far enough

Possible cause: Technical defect

Solution: Service

5 Troubleshooting

```
ERROR 013

Supply pressure is
does not raise

INJECT = Continue
```

Error 13
Supply pressure does not rise

Possible cause: Technical defect

Solution: Service

```
ERROR 014

Supply pressure
leak is too high

INJECT = Continue
```

Error 14
Massive leak in pressure accumulator

Possible cause: Technical defect

Solution: Service

```
ERROR 015

Supply pressure
leak is too high

INJECT = Continue
```

Error 15
Leak in pressure accumulator

Possible cause: Technical defect

Solution: Service

```
ERROR 016

Valve 1 pressure
leak is too high

INJECT = Continue
```

Error 16
Leak through Valve V1 is too large

Possible cause: Technical defect

Solution: Service

```
ERROR 020

Working pressure
cannot be reached

INJECT = Continue
```

Error 20
Working pressure has not been reached

Compressor runs without supply pressure being reached within a specific time.

Possible cause:
Capillary holder not attached or poorly attached. (Broken) capillary with large opening.
Technical defect.

Solution:
Check the capillary. Check the capillary holder and tube for leaks or tighten up the tube connections.
Service.

5 Troubleshooting

```
       ERROR 021

     Inject pressure
    cannot be reached

   INJECT = Continue
```

Error 21
Injection pressure has not been reached

Possible cause:
Capillary holder not attached or poorly attached. (Broken) capillary with large opening.
Technical defect.

Solution:
Check capillary. Check capillary holder and tube for leaks or tighten up tube connections.
Service.

```
       ERROR 022

  Compensation pressure
    cannot be reached

   INJECT = Continue
```

Error 22
Compensation pressure has not been reached

Possible cause:
Capillary holder not attached or poorly attached. (Broken) capillary with large opening.
Technical defect.

Solution:
Check capillary. Check capillary holder and tube for leaks or tighten up tube connections.
Service.

```
       ERROR 023

     Inject n pressure
    overflow too large

   INJECT = Continue
```

Error 23
Increase in working pressure is smaller than required

Possible cause: Technical defect

Solution: Service

```
       ERROR 024

    Working pressure
     does not rise

   INJECT = Continue
```

Error 24
No increase in working pressure

Possible cause: Technical defect

Solution: Service

5 Troubleshooting

```
      ERROR 025

   Working pressure
    does not fall

INJECT = Continue
```

Error 25
No drop in working pressure

Possible cause: Technical defect

Solution: Service

```
      ERROR 026

   Working pressure
    leak is too high

INJECT = Continue
```

Error 26
Working pressure leak rate is too high

Possible cause:
Capillary holder not attached or poorly attached. (Broken) capillary with large opening.
Technical defect.

Solution:
Check capillary. Check capillary holder and tube for leaks or tighten up tube connections.
Service.

```
      ERROR 027

   Working pressure
    leak is too high

INJECT = Continue
```

Error 27
Working pressure leak rate is too high

Possible cause:
Capillary holder not attached or poorly attached. (Broken) capillary with large opening.
Technical defect.

Solution:
Check capillary. Check capillary holder and tube for leaks or tighten up tube connections.
Service.

5 Troubleshooting

ERROR 028

Working pressure
leak is too high

INJECT = Continue

Error 28
Working pressure leak rate is too high

Possible cause:
Capillary holder not attached or poorly attached. (Broken) capillary with large opening.
Technical defect.

Solution:
Check capillary. Check capillary holder and tube for leaks or tighten up tube connections.
Service.

ERROR 030

Sensoroffset supply
is too low

INJECT = Continue

Error 30
Sensor offset supply pressure is too low

Possible cause: Technical defect

Solution: Service

ERROR 031

Sensoroffset supply
is too high

INJECT = Continue

Error 31
Sensor offset supply pressure is too high

Possible cause: Technical defect

Solution: Service

ERROR 032

Sensoroffset work
is too low

INJECT = Continue

Error 32
Sensor offset working pressure is too low

Possible cause: Technical defect

Solution: Service

ERROR 033

Sensoroffset work
is too high

INJECT = Continue

Error 33
Sensor offset working pressure is too high

Possible cause: Technical defect

Solution: Service

ERROR 034

Valve 1 offset
is too low

INJECT = Continue

Error 34
Valve 1 offset is too low

Possible cause: Technical defect

Solution: Service

5 Troubleshooting

```
         ERROR 035

       Valve 1 offset
        is too high

INJECT = Continue
```

Error 35
Valve 1 offset is too high

Possible cause: Technical defect

Solution: Service

```
         ERROR 036

       Valve 2 offset
         is too low

INJECT = Continue
```

Error 36
Valve 2 offset is too low

Possible cause: Technical defect

Solution: Service

```
         ERROR 037

       Valve 2 offset
        is too high

INJECT = Continue
```

Error 37
Valve 2 offset is too high

Possible cause: Technical defect

Solution: Service

```
         ERROR 040

       Self-Calibration
         is missing

INJECT = Continue
```

Error 40
Self-calibration is missing

Possible cause: Technical defect

Solution: Service

```
         ERROR 041

      Fullscale Calibration
          is missing

INJECT = Continue
```

Error 41
Full-scale calibration is missing

Possible cause: Technical defect

Solution: Service

6 Technical data

Voltage/Frequency:	100 V – 240 V $\pm$ 10 %, 50 – 60 Hz
Set voltage:	adapts automatically
Fuse for 100 – 240 V:	T1.0 A, 250 V (2 pcs.)
Power consumption:	50 W
Max. current consumption:	< 0.5 A
Protection class:	I
Overvoltage category:	II (IEC 61010-1)
Pollution degree:	2 (IEC 664)
Ambient temperature:	15 °C – 35 °C
Ambient relative humidity:	max. 70 %
Weight:	6.2 kg
Dimensions:	Width: 220 mm Depth: 170 mm Height: 280 mm
Injection time t_i:	0.0 – 99.9 s in 0.1 s increments
Injection pressure p_i:	0 – 6,000 hPa (87 psi), in 1 hPa (0.01–0.02 psi) increments
Regulating accuracy:	1 % of value set in each case, at least $\pm$ 1 hPa
Compensation pressure p_c:	0 – 6,000 hPa (87 psi), in 1 hPa (0.01–0.02 psi) increments
Regulating accuracy:	1 % of value set in each case, at least $\pm$ 1 hPa
Clean rinsing pressure:	max. approx. 7,000 hPa (102 psi)
RS 232 computer interface:	Baud rate: 9,600 Start bit: 1 Data bits: 8 Parity: non Stop bit: 1

The device is approved with the $C\epsilon$ sign.

Technical specifications subject to change!

7 Ordering information

Order no.

5247 000.013	FemtoJet®
	100 V – 240 V ± 10 %, 50 – 60 Hz
	Fully programmable microinjector with integrated pressure supply. For injection of smallest amounts of liquid into living cells.
	Delivery package:
	1 FemtoJet®
	1 Power cable
	1 Interface cable 5171
	1 Universal capillary holder
	1 Pressure tube, 2 m
5247 617.002	1 Hand control for remote control
	10 Femtotips® / Femtotips® II (injection capillaries)
	10 Microloader
	20 CELLocates
	1 Operating Manual

Accessories (not standard)

5247 623.002	Foot control FemtoJet®
5325 620.007	Interface cable PC for control and for program updates
5247 622.006	Interface cable 5171 for connection to the micromanipulator
5242 952.008	20 Femtotips®
5242 957.000	20 Femtotips® II
5242 956.003	200 Microloader
5246 164.004	1 Pressure tube, 2 m, for universal capillary holder
5176 190.002	Universal capillary holder (incl. grip head 0, adapter for Femtotips®, clamping piece and spare O-rings)
4910 000.018	Eppendorf Reference® pipette, variable, 0.5 to 10 µl
3110 000.021	Eppendorf Research® pipette, variable, 0.5 to 10 µl
5176 210.003	Grip head 0 for universal capillary holder; for microcapillaries with an outer diameter of 1.0 to 1.1 mm
5176 212.006	Grip head 1 for universal capillary holder; for microcapillaries with an outer diameter of 1.2 to 1.3 mm
5176 214.009	Grip head 2 for universal capillary holder; for microcapillaries with an outer diameter of 1.4 to 1.5 mm

7 Ordering information

5176 207.002	Grip head 3 for universal capillary holder for microcapillaries with an outer diameter of 0.7 to 0.8 mm
5176 196.000	O-ring set for grip heads 0 to 3 with extraction tool
5176 195.004	Service kit for grip head and capillary holder, consisting of O-ring set, adapter for Femtotip® and clamping piece

CELLocate
Glass coverslips with integrated microgrid
for relocating cells in light and electron microscopy,
individually sealed, with log pad

5245 952.009	Grid size 175 μm, sterile, set of 80
5245 962.004	Grid size 55 μm, sterile, set of 80

Micromanipulation

5171 000.019	Micromanipulator 5171 Programmable micromanipulator with coordinate storage for automatic microinjection, microdissection and electrophysiology (in combination with FemtoJet®), complete with power unit, module unit and control board
5179 000.018	InjectMan® Micromanipulator for automatic injection in adherent cells in combination with FemtoJet®, complete with power unit, module unit and control board
5178 000.014	TransferMan® NK Proportional micromanipulator for microinjection in suspension cells
5177 000.010	TransferMan® Dynamic micromanipulator for microinjection in suspension cells
5246 621.006	Transmission cable 5246 "Y cable" for connection with the interface cable 5171
5211 058.007	Foot control for microinjection, connectable to transmission cable 5246 "Y cable"

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8 Index

Appendix A

Operation via a computer

Certain commands and queries (Section 6.2) can be transmitted to the unit using the optional cable/PC interface.

A.1 Description of interface

A computer can be connected to the remote control of the FemtoJet® via one of the two RS 232 interfaces. Parameters can be entered or read out via the computer.

The computer is connected by the special cable/PC interface cable.

The computer to be connected must comply with EN 60950/UL 1950 standards.

The serial interface of the computer should be set to the following transmission parameters:

Baud rate: 9,600
Start bit: 1
Dat bits: 8
Parity: non
Stop bit: 1

Only ASCII symbols (incl. CR/LF) which can be represented are transmitted.Therefore a terminal program, for example, is suitable for remote control.

A.2 Commands and queries

The commands to be sent are defined with the following structure:

Commands to enter parameters: "Cxxx=ssssssssss"
Commands to execute and to read out parameters: "Cxxx"
C: Command
xxx: Code for the executing command
=:   Separation for the following input parameters
ssssssssss: Input parameters

In response to any command, the device provides a symbol string of a similar structure:

"Axxx" for answers without output parameter
"Axxx=rrrrrrrrrr" for responses with output parameter
A: Answer

Appendix A

If the command is faulty, an error message is returned instead of the answer:

"ERR=eee"

ERR: Error
=: Separation
eee: Error number

Lower and upper case letters can be used.

Command to enter parameters [C]	*Code* [xxx]	*Input parameter* [ssssssssss]	*Meaning of input parameters*
Short acoustic signal	003	1 – 254	Number of signals
Long acoustic signal	004	1 – 254	Number of signals
Automatic injection (inject at t_i)	010	0; 1 – 999	Injection time in 1/10 seconds 0: injection time set on the FemtoJet®
Set injection counter n to 0	012	– – –	No parameter input
Perform Clean	013	1 – 100	Time in 1/10 seconds
Execute Standby	014	0 or 1	0 = short 1 = long
Switch acoustic signal on/off	040	0 or 1	Status: 0 = off / 1 = on
Set pressure unit	041	0 or 1	Unit: 0 = hPa / 1 = psi
Set injection time t_i	042	1 – 999	Injection time in 1/10 seconds
Set compensation pressure p_c	043	0 – 6,000 / 87	Pressure in hPa/psi
Set injection pressure p_i	044	0 – 6,000 / 87	Pressure in hPa/psi

Appendix A

Command to enter parameters [C]	*Code* [xxx]	*Input parameter* [sssssssss]	*Meaning of input parameter*
Set background illumination	045	0 or 1	0 = off 1 = on
Dewater	046	– – –	No parameter input

Command to read out parameters [C]	*Code* [xxx]	*Significance of output parameter* [A]	*Output parameter* [rrrrrrrrr]
Query program version	101	Versions number	x.xx
Query bleeper	150	Status: 0 = off / 1 = on	0 or 1
Query pressure unit	151	Unit: 0 = hPa / 1 = psi	0 or 1
Query injection time t_i	152	Injection time in 1/10 seconds	1 – 999
Query compensation pressure p_c	153	Pressure in hPa/psi	0 – 6,000 / 87
Query injection pressure p_i	154	Pressure in hPa/psi	0 – 6,000 / 87
Query background illumination	155	Status: 0 = off / 1 = on	0 or 1

Appendix A

Error no. for incorrect operating construction ERR = [eee]	*Cause*	*Solution*
060	The first character is not a c or C.	Check entry parameters. Change entry.
061	The 2nd to 4th character is not a digit.	Check entry parameters. Change entry.
062	The 5th character is not =.	Check entry parameters. Change entry.
063	The parameter is not within the permitted range.	Check entry parameters. Change entry.
064	The command is not known.	Check entry parameters. Change entry.

Appendix A

Error no. during execution ERR = [eee]	*Cause*	*Solution*
070	FemtoJet® is not in routine mode.	Check FemtoJet®. Bring FemtoJet® into routine operation. Eliminate error.
071	FemtoJet® is not ready.	Check FemtoJet® or micromanipulator. As necessary, wait or eliminate error.
072	The command is not finished within the scheduled time.	Check FemtoJet® or micromanipulator. Eliminate error.
073	This command cannot be executed at the moment, because of insufficient storage pressure. See: Warning 001	Check FemtoJet®. Eliminate error.
074	The connected micro-manipulator did not respond. See: Warning 003	Check micro-manipulator. Eliminate error.

Other error numbers as well as the error cause and solution are described in Section 5, "Troubleshooting". During remote control, no error texts appear in the FemtoJet® display.

Support and Services Directory

Contact Information

		United States	Canada
	Business Hours:	8:30 a.m. to 6:00 p.m. EST	8:30 a.m. to 6:00 p.m. EST
	Phone:	800-645-3050 516-334-7500	800-263-8715 905-826-5525
	Fax:	516-334-7506	905-826-5424
	Address:	Eppendorf North America, Inc. One Cantiague Road Westbury, NY 11590-0207	Brinkmann Instruments (Canada) Ltd. 6670 Campobello Road Mississauga, ONT L5N 2L8
	Website: **Email:**	www.eppendorf.com info@eppendorf.com	www.brinkmann.com canada@brinkmann.com

	Customer Support:	800-645-3050, menu option 2 custserv@eppendorf.com	800-263-8715, menu option 1 custserv@brinkmann.com
	Repair:	800-645-3050, ext. 2404 service@eppendorf.com	800-263-8715, ext. 232 service@brinkmann.com
	Applications Lab:	800-645-3050, ext. 2258 apps@eppendorf.com	800-645-3050, ext. 2258 (U.S.) bioapps@brinkmann.com

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