Electroporator 2510

Operating Manual

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EC Conformity Declaration

1 Introduction

The Eppendorf Electroporator is used for the electroporation of bacteria and yeast cells where pulses of very high electrical field strength are applied to samples of small volume and high resistance.

The electroporator contains a capacitor that can be selectively charged to a voltage between 200 and 2,500 volts. This capacitor is then discharged into a resistor to create an exponential discharge curve. The exponential pulse created by the system is applied to a small disposable cuvette containing a biological sample.

The results of the electroporation can be transferred to a printer or a PC.

In contrast to devices from other manufacturers, the Eppendorf Electroporator has an integrated cuvette holder.

The electroporator is easy to use. The device does not have components which have to be maintained by the operator.

The construction of the electroporator minimizes the risk of arcing even in the case of invalid high salt concentrations and maximum voltage. Even in the extremely improbable case of arcing in the cuvette, no bacterial suspension leaves the cuvette and pollutes the device.

The Eppendorf Electroporator corresponds with valid UL regulations and is CE certified.

2 Technical description

2.1 Configuration

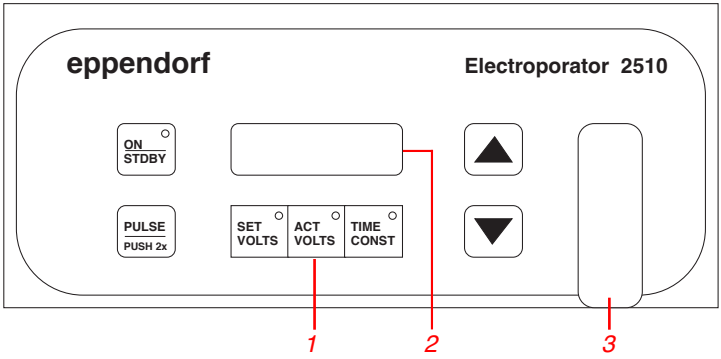


Fig. 1: Schematic diagram of the front panel of the electroporator

1.1 Keys
1.2 Display
1.3 Integrated cuvette holder

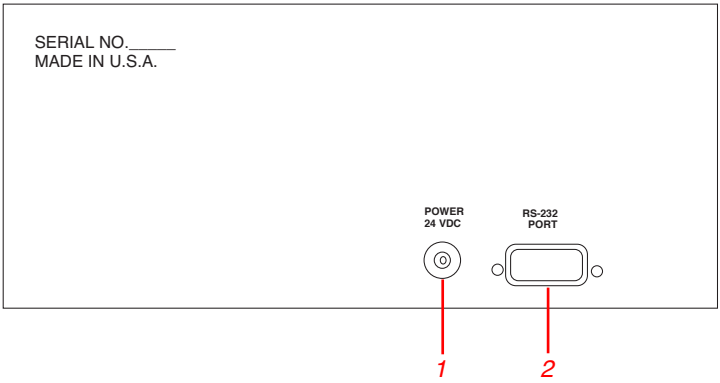


Fig. 2: Schematic diagram of the rear panel of the electroporator

2.1 Main power connection
2.2 Serial interface

2 Technical description

2.2 Display and keys

The electroporator is operated with the keys on its front panel. Settings or outputs are shown on its four-digit display.

The keys have the following functions:

This key switches the electroporator on or into the standby mode. A green LED indicates that the external direct-voltage adapter is supplying power to the electroporator. In standby, no other keys are active and the display is blank.

Pressing this key two times within two seconds starts a charge/discharge cycle.
During charging, the display changes from the set voltage to "Chg".

Pressing any key aborts the charge/discharge cycle and discharges the internal capacitor immediately. When the discharge takes place, an internal speaker beeps to indicate that the electroporation is complete.

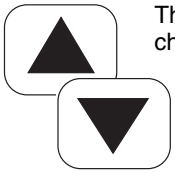
To display the set voltage, press this key. A red LED indicates that the display has been updated to the currently set voltage. The voltage can be modified with the aid of the arrow keys.

To display the actual voltage delivered to the cuvette, press this key. A red LED indicates that the display is showing the last actual peak voltage delivered.

2 Technical description

To display the time constant of the last discharge, press this key. A red LED indicates that the display is showing the last time constant, measured in milliseconds.

The date and time can be set by pressing ***SET VOLTS*** and ***TIME CONST*** simultaneously.



The arrow keys are used to adjust the set voltage and to change the date and time.

2.3 Connections

The connections for the main power supply (Fig. 2.1) and a serial interface (Fig. 2.2) are located on the rear panel of the electroporator.

The connections for the main power supply is marked POWER 24 VDC.

The electroporator can be connected via the serial interface and a DB-9 M/F cable to a printer or a PC.

2.4 Cuvette holder and cuvettes

The integrated cuvette holder (Fig. 1.3) is to the right of the keys. The opening into which the cuvette is inserted is accessible after the cuvette holder has been pulled out.

The biological sample is presented in a disposable cuvette which is rectangular and sealed with a lid. Its upper half is made of plastic. In the lower half of the cuvette, two parallel surfaces are limited by plastic, the other two surfaces by plate electrodes.

The gap between the electrodes depends on the cuvette type:
1 mm (= 100 µl filling volume), 2 mm (= 400 µl filling volume) or
4 mm (= 800 µl filling volume).

2 Technical description

The width of the gap and the filling volume are stated on the cuvettes.

A plastic nose located on one of the two plastic surfaces in the lower part of the cuvette ensures that the cuvette is correctly inserted into the cuvette holder.

A frosted window enables labelling of the cuvette.

The cuvettes are sterilized with gamma rays and are individually sealed.

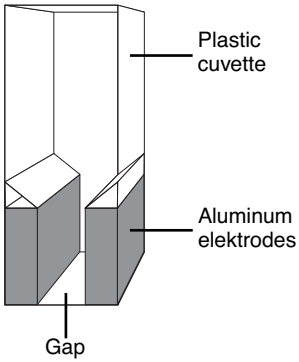


Fig. 3: Schematic diagram of the electroporation cuvette

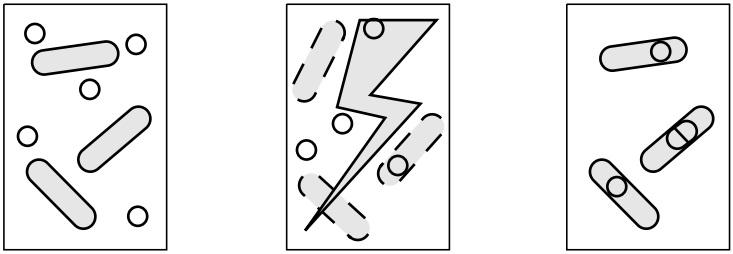
3 The principle of electroporation

Electroporation is an efficient means of transferring macromolecules such as DNA into bacteria or yeast strains.

Electroporation is the process of applying high voltage electric field pulses of short duration to create temporary holes or pores in the membrane of cells. These pores are generally large enough to allow macromolecules to diffuse into the cell.

Upon removal of the electric field and a period of recovery, these pores reseal and the DNA is free to become transcribed and replicated within the cell.

Electroporation produces results equivalent to, if not superior to, chemical means of transferring macromolecules into cells. In addition, electroporation is often an easier protocol to perform.



DNA and bacteria

Electrical discharge 2,500 V 5 ms

DNA in bacteria

Fig. 4: Principle of electroporation

4 Safety precautions

The electroporator is CE certified and thus meets the relevant fundamental safety and health requirements of the following EC guidelines:

– EN 61010-1	(Electrical safety)
– EN 55011/ B, EN 500821	(Interference suppression)
– EC 801-2 801-3, 801-4, 801-5, 801-6	(Noise immunity)
– IEC 1000-4-11	(Noise immunity)

Caution:

The Electroporator 2510 is a high voltage instrument. No user serviceable parts are contained inside. If the instrument is not functioning as described, please contact Eppendorf for assistance.

The Eppendorf Electroporator 2510 is not meant for use with samples of low resistance (buffered saline solutions or buffered sucrose solutions). It is imperative that samples be prepared properly in a high electrically resistant medium with all salts removed for the Electroporator 2510 to function properly.

Even in the case of arcing the design of Eppendorf cuvettes prevent any bacterial suspension from polluting the device.

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.

5 Operating the electroporator

5.1 Performing electroporation

– Switching on the electroporator.

Plug the power unit into the power connection marked POWER 24 VDC on the rear panel of the electroporator and connect to an electrical outlet.

When the main power connection has been established, the electroporator is switched on. The software version is displayed followed by a voltage of 0 V.

Sample counting begins after switch-on with sample number 1.

– Filling and inserting the cuvette.

Pipette bacteria and concentrated DNA plasmid solution into a cuvette. There must not be any bubbles in the gap between the plate electrodes. Insert the cuvette into the cuvette holder of the electroporator so that the plastic nose of the cuvette fits into the slit of the cuvette holder. Push the cuvette holder into the electroporator until it clicks into position.

– Selecting the voltage.

After the cuvette has been inserted into the cuvette holder, select a voltage between 200 and 2,500 with the aid of the SET VOLTAGE and arrow keys.

After the electroporator has been switched on, a voltage of 0 V is displayed. After the arrow key ▲ has been pressed, the voltage jumps to 200 V and each time this key is pressed again, it is increased by 10 V. Each time the arrow key ▼ is pressed, the voltage is reduced by 10 V.

In addition, it is possible to select the voltage values used most frequently, i.e., 1,800 V and 2,500 V, directly by pressing both arrow keys simultaneously. The voltage first jumps from 0 to 1,800 V, then from 1,800 to 2,500 V and then back to 0 V.

5 Operating the electroporator

– Starting the charging/discharging procedure.

  The charging/discharging procedure is started by pressing the PULSE key twice. During charging (approx. 8 seconds at maximum voltage), "Chg" appears in the display. After discharge, the unit will beep signaling that electroporation is concluded. The display switches back to the set voltage.

  The charging/discharging procedure can be aborted at any time by pressing any key.

– Removing the sample.

  The cuvette is removed from the cuvette holder. The sample is transferred into medium (see Sec. 6.6).

– Querying the last voltage applied and the time constant.

  The voltage last applied at the cuvette can be queried by pressing the ACT VOLTS key. The time constant of the discharge curve (default value: 5 ms) is queried by pressing the TIME CONST key.

– Changing over to standby operation.

  Press the ON/STDBY key to change over to standby operation. In standby operation, the keys are not active and the display is empty.

5.2 Setting the date and time

Press SET VOLTS and TIME CONST simultaneously to start the time/ date setting mode. This steps through the year, month, date, hour and minute.

The set year is displayed first, e.g. "y 95". The year can be changed with the aid of the arrow keys. Press TIME CONST when set.

The display then jumps to the set month (e.g. "n 2"), after modification and/or confirmation with TIME CONST to the set day (e.g. "d 20"), then to the set hours (e.g. "h 17") and finally to the set minutes (e.g. "= 30").

Confirming the minute display completes the time/date setting.

5.3 Connecting a printer or computer

The electroporator can be connected to a printer with an RS-232 interface or a PC with a standard DB-9 M/F cable via the serial interface located on its rear panel (RS-232).

The results printed out or transferred to the PC include a sample number, the date and time of electroporation, the set voltage (SET), the actual voltage (ACT) and the time constant of the discharge curve (TC).

The sample number is incremented during each charge/discharge cycle and reset to sample #1 when the device is turned off.

An example printout looks like this:

```
Sample #1       2/20/95 15:20
SET=1500 ACT=1500 TC=5.8

Sample #2       2/20/95 15:35
SET=2500 ACT=2450 TC=5.8

Sample #3       2/20/95 16:05
SET=1000 ACT=1000 TC=5.8

Sample #4       2/20/95 16:15
SET=2000 ACT=1970 TC=5.6
```

6 Factors influencing electroporation

There are a number of factors that affect the success of electroporation. These include the quality and concentration of DNA being introduced, the resuspension medium of both the DNA and the cells, the strain of bacteria or yeast being electroporated and the electroporation conditions.

6.1 DNA preparation

It is very important to reduce the amount of ions and other contaminants present in the DNA preparation. Common sources of residual ions in DNA solutions are cesium chloride from plasmid preparations and ammonium acetate from ethanol precipitations.

DNA dissolved in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) is acceptable as long as this DNA is diluted approximately 10 fold with the electro-competent cell suspension.

DNA may also be directly electroporated from ligation mixtures and enzyme reactions, if the final salt concentration is below 5 mm or its equivalent. If the reaction mixture's ionic strength is too high, it may be reduced by dilution[1)] or by ethanol precipitation[2, 3)]. After ethanol precipitation, the DNA may be resuspended in double distilled water or TE.

If the DNA is to be stored for an extended period of time, resuspension in TE is advisable.

Electroporation of *E.coli* allows a variety of plasmid sizes and topologies to be successfully transformed. It has been reported that plasmids of 21 kb transform *E.coli* with the same molar efficiency as plasmids of 3 to 7 kb[4)]. Supercoiled and relaxed circular forms of plasmids up to at least 20 kb transform with similar efficiencies, while linear DNA has been shown to be about 10^4-fold less active than the same DNA transformed in circular form. These data may be very different for various strains and circumstances. Often, optimum conditions will need to be worked out empirically.

It is important not to add the DNA to the cell suspension too soon before electroporation. Generally, the DNA is added to the cells one minute before electroporating and kept at 0 °C (e.g. Eppendorf IsoTherm System). This is done to avoid DNA degradation due to any DNases in the cell suspension which may reduce the efficiency of the electroporation.

6 Factors influencing electroporation

The concentration of DNA may also greatly affect the recovery of transformants. There are two measurements of concern with transformations: frequency (transformants/survivor) and efficiency (transformants/μg DNA).

The frequency of transformation is dependent on DNA concentration over at least six orders of magnitude (10 pg/ml up to 7.5 μg/ml). There have been 80 % frequencies in the 7.5 μg/ml range reported[5]. Since the number of transformants recovered is the product of the frequency and the number of cells present, efficiency of transformations increases with an increase in cell concentration over the range from 10^9 to at least 3×10^{10} cells/ml. High frequencies may be obtained by using high DNA concentrations, while high efficiencies are obtained by using high cell concentrations.

Lowering the DNA concentrations will help to avoid co-transformations of the same cell.

6.2 Electroporation medium

Since electroporation creates temporary pores in cell membranes, it is important to remember that the cells are exposed to their external environment and any contaminants which may be present.

During electroporation, electrolysis of the medium drastically affects the characteristics of the medium (i.e. the pH). Many electroporated cells will die if fresh medium is not immediately introduced for recovery. Typically a rich medium for recovery is best, such as SOC medium (0.5 % yeast extract, 2 % tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 20 mM $MgSO_4$, 20 mM glucose) for *E.coli.*

The ionic strength of the medium is an important consideration when using high voltage electroporators. It is very important to keep the resistance of the medium as high as possible by making sure salts have been removed from the cell and DNA preparations (described under DNA preparation). A common source of residual ions in the cell suspension is the culture medium.

Higher efficiencies will be obtained by removing salts from both the DNA solution and the cell preparations. Generally, the lowest possible ionic solution that the cells will withstand is preferable.

6 Factors influencing electroporation

6.3 Growth and preparation of cells

Although there is no general agreement on the appropriate cell cycle for harverst of all strains, *E.coli* conditions have been well defined.

E.coli bacterial strains typically used for electroporation are harvested while in exponential growth phase for maximum efficiency[4]. For some strains, late exponential or stationary-phase cells perform best[6, 7].

For all cell types, thorough washing of the cells to remove growth medium during electro-competence protocols is critical.

The final concentration of the cells should be approx. 1–3 x 10^{11} cells/ml.

6.4 Electroporation conditions

Each strain of bacteria or yeast will have optimal conditions that will need to be empirically determined. These conditions include the volume of cells, the amount of specific plasmid of interest and the field strength applied. Generally, for *E.coli*, field strengths of 12–19 kV/cm are required to obtain maximum transformation efficiency.

6.5 Temperature

Electroporation at low temperatures (0–4 °C) generally works best. This may be accomplished by equilibrating the cuvettes to 0 °C before electroporation. The cuvette holder may also be cooled before electroporation.

After chilling, blot off the excess ice or moisture from the cuvette and holder before inserting into the electroporator.

6.6 Immediate transfer for recovery

Electroporation of cells causes the electrolysis of the medium and electrodes. This change in the medium is not favorable for the cells. It is therefore very important to resuspend the cells into fresh medium (without selection chemical) as soon as they have been electroporated. Quick resuspension in approximately 1 ml helps to minimize cell death. The cells should be allowed to recover at 37 °C for 1 hour shaking at 225 rpm (or whatever conditions were used for optimal growth of the strain).

6.7 Selecting transformants

Once the cells have recovered, they should be grown on selective plates.

To determine efficiency, plate various concentrations of cells and calculate the number of transformants/µg DNA.

7 Toubleshooting

7.1 Operating errors

– The display does not light up when the ON/STDBY key is pressed.

  Check the power supply to the electroporator (power cable and transformer).

– "Chg" does not appear in the display when the PULSE key is pressed twice.

  The key was not pressed twice within 2 seconds.

– The transformation efficiency is too low.

  The cuvette holder was not inserted far enough into the device to make contact with the electrodes.
  The medium in the cuvette should appear somewhat lysed after the pulse. This lysate is easy to see when recovery medium is added with a pipette.

7.2 Electrical errors

– The voltage applied to the cuvette varies from the set voltage.

  The resistance of the medium may affect the voltage applied to the cuvette. A great variance may indicate that the resistance of the medium is too low and that arcing may have occurred. Arcing will not cause damage to the device, but will affect your experiment.

  There are a number of causes that may contribute to low resistance:

  - Washing and resuspending cells in a buffer too high in ionic strength.
  - Insufficient washing of the cells in preparation. Any growth medium carried over between washes will leave behind unwanted salts.
  - Lysed cells in the preparation. The cell contents contribute to lowering the resistance of the medium.
  - The DNA preparation is too high in salt.
  - Time constant is not as expected (5 ms)

    If a lower time constant is noted, this may be due to a low resistance of the medium (for causes see above).

7.3 Biological errors

– Transformation efficiency is too low to be useful.

There are many factors that may contribute to low transformation efficiencies:

- ***The pulse***
  Each strain of bacteria has optimal conditions. There should be some cell death with electroporation. If the field strength is too high or low, transformation efficiencies will be low. Expected survival rates range from 20 to 80 %.
  E. coli require pulses of approximately 5 ms and field strengths of 12 to 19 kV/cm. To optimize the conditions, test for transformation efficiencies by varying the voltages.

- ***The cells***
  Cells seem to transform best when harvested in early- to mid-log phase.
  Different growth conditions may improve transformation efficiencies.
  If too many cells are killed, optimize the conditions of electroporation for the strain and check for toxic or organic substances in both the DNA and cell preparations.

  Cells (especially *E.coli*) must be transferred immediately to rich medium after pulsing for best results. Even a slight delay may cause a greatly reduced transformation efficiency.

7 Toubleshooting

- ***The DNA***
  Check the quantity and quality of the DNA being used. If the concentration is incorrect or the DNA has been degraded, the transformation efficiency will be low.

  Be sure the DNA preparation is free of salts or other components used in its preparation which may be toxic to the cells.

  Do not add the DNA to the cell solution more than a minute before electroporating. Any DNases in the cell solution may degrade the DNA of interest and cause low transformation efficiencies.

- ***The temperature***
  Chilled cuvettes (0–4 °C) work better than room temperature cuvettes. Be sure to prechill the cuvettes before using.

  If frozen, the cells should be used soon after they are thawed. Frozen cells should be stored at -70 ˚C in 10–15 % glycerol for a maximum of 6 months to a year.

8 Technical data

Power supply:	24 V ± 20 % direct voltage (DC), 0.5 A max., from external power unit
Set voltage:	200 to 2,500 V
Capacitor:	10 µF, 2,500 V pulse discharge
Resistor:	600 Ohms
Charge time:	< 8 seconds
Discharge time constant:	5 ms nominal with a 3.3 kOhm sample impedance
Display:	four-digit
Displayed values:	Set voltage Actual peak voltage Time constant
Dimensions:	Width: 22 cm Height: 10 cm Depth: 27 cm
Weight:	2.8 kg
Date/Time:	Internal calendar/real-time clock
Interface:	RS 232 Communication parameters: 4,800 bauds no parity 8 data bits 1 stop bit Plug: DB-9 standard plug with following pin allocation:

Pins 1, 4 and 6	connected
Pins 7 and 8	connected
Pin 5	ground
Pin 2	data from electroporator (tx)
Pin 3	data to electroporator (rx)

9a Ordering information

Order no.	
4307 000.658	Electroporator 2510, 230 V (for use in Europe), including 10 sterile cuvettes with a 1 mm gap
	Electroporation cuvettes
4307 000.569	1 mm electrode gap, sterile, set of 50
4307 000.593	2 mm electrode gap, sterile, set of 50
4307 000.623	4 mm electrode gap, sterile, set of 50
4308 078.006	Cuvette stand for 16 electroporation cuvettes
4307 000.771	Power unit for 230 V (for use in Europe)
4307 000.275	Cuvette holder for Electroporator 2510
4307 900.012	Operating manual

9b Ordering information for North America

Order no.	
940000009	Electroporator 2510, 110 V including 10 sterile cuvettes with a 1 mm gap
940000017	Electroporator 2510, 230 V including 10 sterile cuvettes with a 1 mm gap
	Electroporation cuvettes
940001005	1 mm electrode gap, sterile, set of 50
940001013	2 mm electrode gap, sterile, set of 50
940001021	4 mm electrode gap, sterile, set of 50
940001102	Cuvette stand for 16 electroporation cuvettes

Appendix

1 Glossary of terms

Arcing
If an electric field is applied between parallel electrodes, a current will flow in an evenly distributed sheet. If the field exceeds a critical value, the sheet will be restricted to a very narrow channel with a high current density which is called an arc. The electrode material will melt at this spot and vapor will be generated explosively. As a result, the electrodes will show craters or pitting marks. Under these conditions, the cuvette can be destroyed.

Capacitance
The ratio of the charge on one of the plates of the capacitor to the potential difference between the plates.

Capacitor
A device that stores energy in the form of an electric field.
It consists of two metal plates insulated from each other by a dielectric (insulating material, air, gas or vacuum). In an ideal capacitor, no conduction current flows between the plates.
Capacitors can be fixed or variable.

Electric field strength
The potential difference between two electrode points (in V) divided by the distance between the electrodes, called gap, and expressed in cm.
Field strength is expressed as V/cm or kV/cm.
This is true only if the electric field is homogeneous as it is in parallel plate electrodes.

Exponential discharge curve
The waveshape formed when a capacitor is discharged after a pulse is delivered.

Farad (F), microfarad (µF)
The unit of capacitance, 1 µF = 10^{-6} F

Resistance
The property of an electrically conductive material that causes a portion of the energy of an electric current to be converted into heat.

Time constant
The time constant states the time (in ms) in which the voltage has faded to the value U/e.

Voltage
The difference of electric potential between two points expressed in volts or kilovolts.

Appendix

2 Bibliography

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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:

Electroporator 2510

Produkttyp, Product type:

Gerät zur Elektrotransformation von Bakterien und Hefen /

Device for electrotransformation of bacteria and yeast

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

73/23/EWG, EN 61010-1

89/336/EWG, EN 55011/B, EN 61000-6-1, EN 61000-3-2, EN 61000-3-3

Vorstand, Board of Management:

Projektmanagement, Project Management:

07.08.2003

Hamburg, Date:

ISO CERTIFIED 9001

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