npi
Electronic Instruments
for the Life Sciences

made to measure

OPERATING INSTRUCTIONS AND SYSTEM DESCRIPTION FOR THE

ELECTROPORATOR

ELECTROPORATION AMPLIFIER

±100 V / ±10 mA max. (current + voltage version)

VERSION 1.6
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1. Safety Regulations

<u>VERY IMPORTANT</u>: Instruments and components supplied by npi electronic are NOT intended for clinical use or medical purposes (e.g. for diagnosis or treatment of humans) or for any other life-supporting system. npi electronic disclaims any warranties for such purpose. Equipment supplied by npi electronic must be operated only by selected, trained and adequately instructed personnel. For details please consult the GENERAL TERMS OF DELIVERY AND CONDITIONS OF BUSINESS of npi electronic, D-71732 Tamm, Germany.

1) GENERAL: This system is designed for use in scientific laboratories and must be operated by trained staff only. General safety regulations for operating electrical devices should be followed.

2) AC MAINS CONNECTION: While working with the npi systems, always adhere to the appropriate safety measures for handling electronic devices. Before using any device, please read manuals and instructions carefully.

   The device is to be operated only at 115/230 Volt 60/50 Hz AC. Please check for appropriate line voltage before connecting any system to mains.

   Always use a three-wire line cord and a mains power-plug with a protection contact connected to ground (protective earth).

   Before opening the cabinet, unplug the instrument.

   Unplug the instrument when replacing the fuse or changing line voltage. Replace fuse only with an appropriate specified type.

3) STATIC ELECTRICITY: Electronic equipment is sensitive to static discharges. Some devices such as sensor inputs are equipped with very sensitive FET amplifiers, which can be damaged by electrostatic charge and must therefore be handled with care. Electrostatic discharge can be avoided by touching a grounded metal surface when changing or adjusting sensors. **Always turn power off when adding or removing modules, connecting or disconnecting sensors, headstages or other components from the instrument or 19” cabinet.**

4) TEMPERATURE DRIFT / WARM-UP TIME: All analog electronic systems are sensitive to temperature changes. Therefore, all electronic instruments containing analog circuits should be used only in a warmed-up condition (i.e. after internal temperature has reached steady-state values). In most cases a warm-up period of 20-30 minutes is sufficient.

5) HANDLING: Please protect the device from moisture, heat, radiation and corrosive chemicals.

2. Special Safety Notice for High Voltage Instruments

HIGH VOLTAGE!!
RISK OF ELECTROCUTION!!

Observe extreme caution when working with this instrument!!!

1) Always connect high voltage power supplies to protective earth!!
2) Do not touch connections unless the instrument is turned off and the capacitance of both the load and power supply are earthed!!
3) Allow adequate time for discharge of internal capacitance of the power supply!!
4) Do not ground yourself or work under wet or damp conditions!!
5) Servicing should be only done by qualified personnel aware of the hazards!!
6) If in doubt, return to supplier for servicing!!

3. ELP-02D ELectroPORATOR Unit

3.1. System Description

The ELectroPORATOR unit is designed for application of voltage pulses for electroporation of cells. The output signal is not isolated from ground and is a voltage up to ±100 V with a current up to ±10 mA.

The ELectroPORATOR will reproduce a waveform applied to its SIGNAL INPUT according to the scaling chosen on the OUTPUT SCALING switch.

The output voltage or resistance can be read at the displays of the device, the applied waveforms can be monitored through BNC connectors at the rear panel by using an oscilloscope or a data acquisition system.

The output at the connected electrode can be corrected by the OFFSET POTENTIAL control (if displays or oscilloscope show not the expected values).

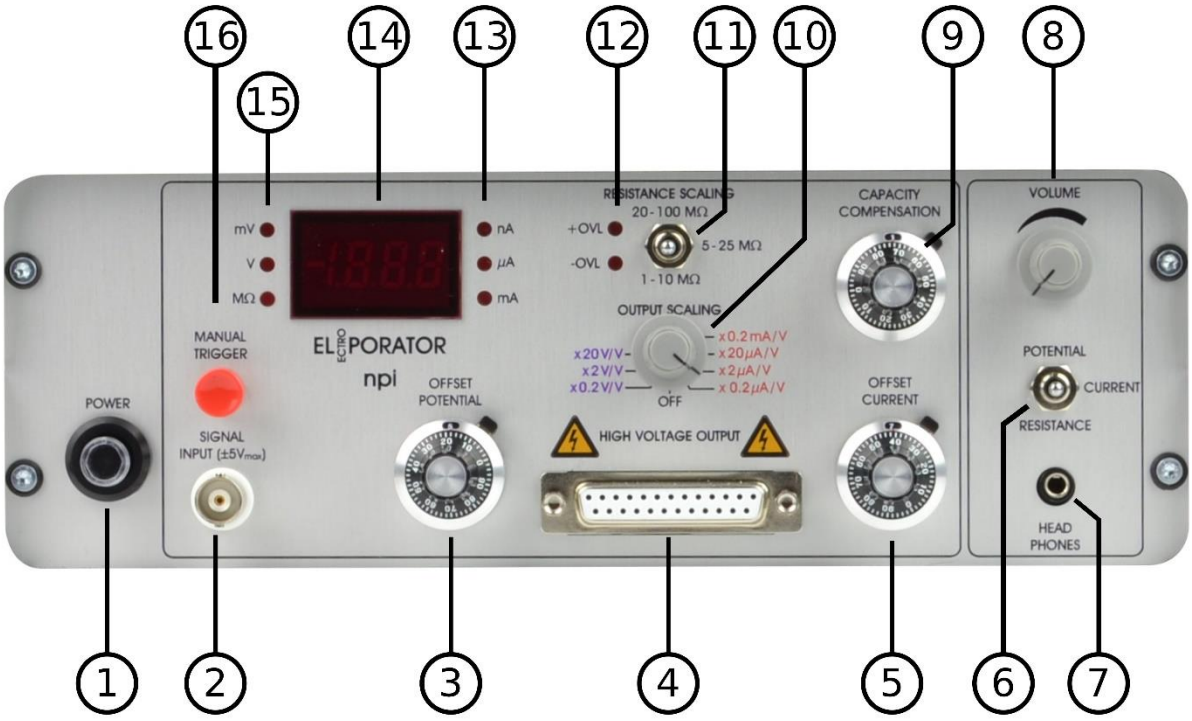

Figure 1: ELectroPORATOR front panel view

Important: The input voltage at SIGNAL INPUT (**#2**) must not exceed ±5 V

3.2. Description of the Front Panel

(**1**) POWER switch

Pushbutton to switch the amplifier ON (pushed) or OFF (released).

(**2**) SIGNAL INPUT (±5 Vmax) connector

BNC connector for the input of an analog signal. Please do not exceed ±5 V.

(**3**) OFFSET POTENTIAL potentiometer

Potentiometer to compensate for the potential offset of the electroporation output. It is recommended to compensate the offsets only in a completely warmed up condition i.e. after 45 to 60 minutes warm-up time. Ranges are dependent on OUTPUT SCALING. See tables in chapter Technical Data.

Note: This offset potentiometer compensates only the offsets in the output voltage source. It is only functional in POTENTIAL OUTPUT mode (see **#10**).

(**4**) HIGH VOLTAGE OUTPUT connector

The headstage cable is connected here (Sub-D 25 connector).

(**5**) OFFSET CURRENT potentiometer

Potentiometer to compensate for the current offset of the electroporation electrode. It is recommended to compensate the offsets only in a completely warmed up condition i.e. after 45 to 60 minutes warm-up time. Ranges are dependent on OUTPUT SCALING. See tables in chapter Technical Data.

Note: This offset potentiometer compensates only the offsets in the output current source. It is only functional in CURRENT OUTPUT mode (see **#10**).

Important: The selected OUTPUT SCALING (see **#10**) has to match the resistance of the electrode in use. Otherwise, the current output might go into saturation, sending out a large current to the electrode.

Rule of thumb: high resistance → low current range, low resistance → high current range.

(**6**) POTENTIAL/CURRENT/RESISTANCE switch

This switch selects whether the POTENTIAL, CURRENT or the RESISTANCE is audible with the AUDIO MONITOR. Furthermore, it activates RESISTANCE mode.

Note: This switch will put the device into RESISTANCE mode: small voltage pulses are applied to the electrode and the resulting current changes are measured. These values are then transferred to the AUDIO MONITOR and the DISPLAY.

(**7**) HEADPHONES connector

3.5 mm headphone connector providing the audio monitor signal. The internal loudspeaker is disabled if a headphone is connected.

(**8**) VOLUME potentiometer

Potentiometer for adjusting the output volume for the internal loudspeaker and the headphone jack #9.

(**9**) CAPACITY COMPENSATION potentiometer

Potentiometer to compensate for the stray capacitances of cables and electrodes in CURRENT output mode. It is recommended to compensate the capacity only in a completely warmed up condition i.e. after 45 to 60 minutes warm-up time. See also chapter 4.3.

Note: For higher impedances, it might be impossible to compensate the capacitance completely. The necessary currents and voltages might exceed the maximum output range of the instrument.

(**10**) OUTPUT SCALING switch

8-position switch for selecting how the output signal is scaled relative to the input signal at **#2**. There are 3 ranges for potential output mode and 4 ranges for current output mode.

OUTPUT SCALING	**Maximum output**	**OFFSET range**
x0.2 V/V	1 V	± 10 mV
x2 V/V	10 V	± 100 mV
x20 V/V	100 V	± 1 V
OFF	-	-
x0.2 µA/V	1 µA	± 10 nA
x2 µA/V	10 µA	± 100 nA
x20 µA/V	100 µA	± 1 µA
x0.2 mA/V	1 mA	± 10 µA

(**11**) RESISTANCE SCALING switch

3 position switch to pre-select the electrode resistance measurement range (1-10 MΩ, 5-25 MΩ, 20-100 MΩ).

(**10**) ±OVL LEDs

These LEDs light up if the electrode resistance is too high (+OVL) or too low (-OVL) for the set RESISTANCE SCALING range.

(**13, 14, 15**) POTENTIAL/CURRENT/RESISTANCE display with unit LEDs

This display shows either POTENTIAL or CURRENT (depending on switch #10). In RESISTNACE mode it will display the measured resistance. The resolution depends on setting of OUTPUT SCALING switch. The corresponding unit (mV, V, nA, µA, mA or MΩ) will be indicated by LEDs next to the display. See also technical data.

(**16**) MANUAL TRIGGER button

Button for triggering the application mode when approaching a cell in RESISTANCE mode (voltage or current output, depending on switch **#10**). Pushing this button will also send a TTL output to the TRIGGER SIGNALS OUTPUT TTL BNC (**#5**) at the rear panel.

3.3. Description of the Rear Panel

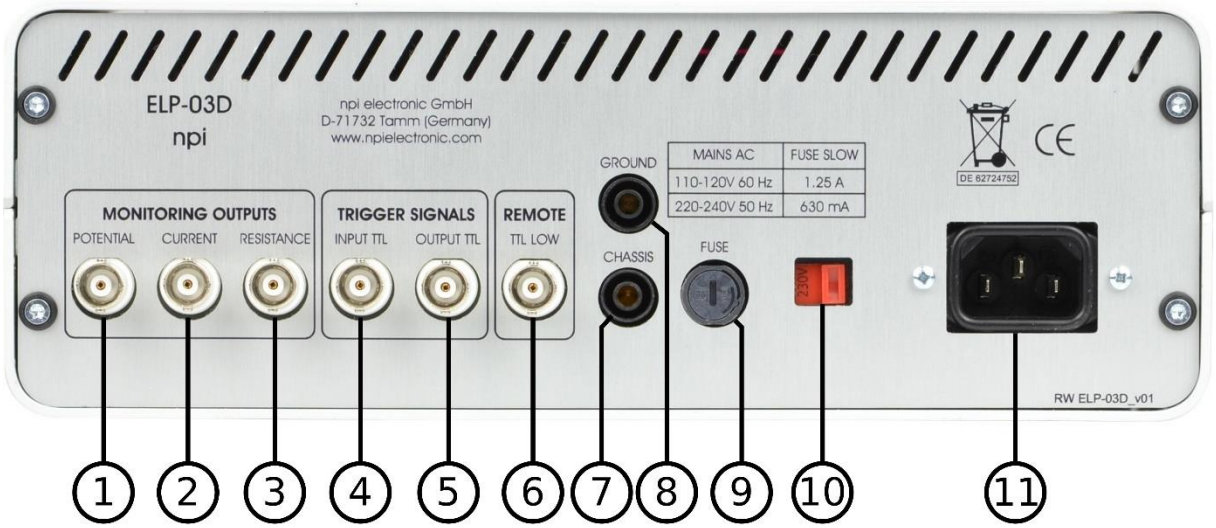

Figure 2: ELectroPORATOR rear panel view

MONITORING OUTPUTS connectors

(**1**) POTENTIAL: scaling 0.1 V/V

(**2**) CURRENT: scaling see table in chapter Technical Data.

Note: this output is only active in CURRENT OUTPUT mode.

(**3**) RESISTANCE: scaling see table in chapter Technical Data.

TRIGGER SIGNALS connectors

(**4**) INPUT TTL

BNC connector for the input of a TTL external trigger signal for triggering the function generator or data acquisition system connected to SIGNAL INPUT.

(**5**) OUTPUT TTL

BNC connector providing a TTL HIGH signal (length: 1 ms) when the electroporator's trigger unit is activated. This TTL signal is used for triggering the function generator or data acquisition system.

(**6**) REMOTE connector

BNC connector with active low logic. A passive footswitch or a remote button can be connected here.

(**7**) CHASSIS connector

Banana plug providing mains ground (see below).

(**8**) GROUND connector

Banana plug providing internal ground (see below).

(**9**) FUSE holder

Holder for the line fuse. For changing the fuse rotate the holder counter-clockwise using a screw driver.

(**10**) LINE SELECT switch

Switch for selecting the line voltage. Switch to the right for 230 V AC, to the left for 115 V AC. The selected voltage is indicated on the switch.

Caution: Before turning on the instrument, make sure that the correct line voltage is selected.

(**11**) Mains connector

Plug socket for the mains power-plug.

Important: Check line voltage before connecting the ELectroPORATOR to power. Always use a three-wire line cord and a mains power-plug with a protection contact connected to ground. Disconnect mains power-plug when replacing the fuse or changing line voltage. Replace fuse only by appropriate specified type (one spare fuse is supplied). Before opening the cabinet unplug the instrument.

3.4. Description of the headstage

Figure 3: Headstage of the ELectroPORATOR.

(1) P_{EL} BNC connector

The pipette holder with the micropipette for electroporation is connected here.

(2) GND connector

2.4 mm connector for connecting the bath electrode.

Important: This headstage is sensitive to electrostatic damage. Switch off the amplifier and make yourself isopotential to the headstage before changing electrodes.

3.5. Description of the model cell

This model cell mimics several different electrode resistances (1, 5, 10, 20 50 and 90 MΩ). The user can switch between these resistances using a 6-position rotary switch. The model cell is connected to the headstage's BNC input connector using the provided adapter.

The use of this model cell allows the user to get familiar with the audio monitor and the different resistance ranges that can be set. There is also a capacitor of 100 pF which will be put in parallel to the chosen resistor if the CAPACITOR switch is in ON position.

Figure 4: Model cell for the ELectroPORATOR.

4. Operation

4.1. General

The instrument is operated by connecting a signal generator or data acquisition analog output to SIGNAL INPUT. This signal can have a maximum range of ±5 V. This signal is transformed into an output signal at HIGH VOLTAGE OUTPUT and scaled according to the settings at the OUTPUT SCALING switch.

The ELectroPORATOR has a trigger unit for starting the function generator or data acquisition system connected to SIGNAL INPUT. This trigger can be set manually by the MANUAL TRIGGER or a footswitch/button connected to REMOTE LOW ACTIVE TTL at the rear panel. It can also be set externally by connecting an external trigger to TIGGER INPUT (TTL). Activating the trigger unit leads to a TTL HIGH signal at TIGGER OUTPUT (TTL).

Unless the instrument is in RESISTANCE mode, any signal connected to SIGNAL INPUT will be transferred into an output signal at the headstage's BNC connector.

4.2. Offset compensation

Before using the ELectroPORATOR compensate for POTENTIAL OFFSET or CURRENT OFFSET – depending on the stimulation mode in use. Output signals can be monitored by connecting an oscilloscope or a data acquisition system to the monitoring outputs on the rear panel.

Note: This offset potentiometer compensates only the offsets in the output source. OFFSET CURRENT is only functional in CURRENT OUTPUT mode, OFFSET POTENTIAL is only functional in POTENTIAL OUTPUT mode (see **#10**).

Important: The selected OUTPUT SCALING (see **#10**) has to match the resistance of the electrode in use. Otherwise, the current output might go into saturation, sending out a large current / voltage to the electrode.

Rule of thumb: high resistance → low current range, low resistance → high current range.

4.3. Capacity compensation

When applying stimuli in current mode, capacitances of cable and pipette will filter the output signal and thereby slow down the system. To overcome this, a capacity compensation circuit is built into the Electroporator.

To adjust the capacity compensation circuit, apply a test pulse with the connected pipette in the bath. Then adjust the CAPACITY COMPENSATION potentiometer until the pulse appears as square shaped as possible. Careful: overcompensation might lead to oscillation.

Note: For higher impedances, it might be impossible to compensate the capacitance completely. The necessary currents and voltages might exceed the maximum output range of the instrument.

4.4. Approaching cells in RESISTANCE mode

RESISTANCE mode (switch **#5**) can be used in combination with the audio monitor for approaching cells. When a cell is found and the pipette gets close to its membrane, this can be heard as a change in resistance.

To cover a wide range of electrode resistances and the large difference in resistance between bath, tissue and cell proximity, the resistance measuring circuit has several ranges, if the measuring range is exceeded or undercut, the unit indicates this with "+/-OVER" LEDs and the user switches one range further.

Triggering the system (by FOOTSWITCH, MANUAL TRIGGER or INPUT TTL) when the pipette tip is close to the cell will change the mode of the amplifier from resistance measurement to application (depending on the setting at the OUTPUT SCALING switch **#8**) for as long as the triggering signal is present. This also sends a signal to the TRIGGER OUTPUT. After releasing the triggering signal, the amplifier immediately returns to resistance measurement mode.

4.5. Approaching cells in POTENTIAL or CURRENTmode

POTENTIAL mode or CURRENT mode (see switch **#6**) can also be used for approaching cells. Triggering the system in this situation will only send a trigger to the TRIGGER OUTPUT, since the amplifier already is in voltage mode or current mode, respectively, and ready for stimulation.

5. Technical Data

Output modes: Voltage or current source

Scaling: For output scaling see table below.

Display: See also table below.

Potential:

OUTPUT SCALING	DISPLAY
x0.2 V/V	XXXX mV
x2 V/V	XXX.X V
x20 V/V	XXX.X V

Current:

OUTPUT SCALING	DISPLAY
x0.2 µA/V	XXXX nA
x2 µA/V	XXX.X µA
x20 µA/V	XXX.X µA
X0.2 mA/V	XX.XX mA

Resistance:

RANGE	DISPLAY
1 – 10 MΩ	XXX.X MΩ
5 – 25 MΩ	XXXX MΩ
20 – 100 MΩ	XXXX MΩ

Trigger Unit: INPUT TTL: active high, 10 kΩ, max. 2 kHz
REMOTE: TTL, active low, 10 kΩ, max. 2 kHz
OUTPUT TTL: active high, 50 Ω, length: 1 ms
Push button

Offset Ranges:

Potential:

OUTPUT SCALING	POTENTIAL OFFSET range
0.2 V/V	±10 mV
2 V/V	±100 mV
20 V/V	±1 V

Current:

OUTPUT SCALING	CURRENT OFFSET range
0.2 µA/V	±10 nA
2 µA/V	±100 nA
20 µA/V	±1 µA
0.2 mA/V	±10 µA

Monitors Scaling:

Potential: 100 mV / V

Resistance: 100 mV / MΩ

Current: Range x0.2 µA / V: 0.1µA / V

Range x2 µA / V: 1µA / V

Range x20 µA / V: 10µA / V

Range x0.2 mA / V: 100µA / V

Resistance Measurement: rectangular voltage signal (±40 mV @ 75 Hz)

Ranges: 1-10 MΩ, 5-25 MΩ, 20-100 MΩ

Input voltage range: ±5 V

Max. output voltage: ±100 V max.

Max. output current: ±10 mA max.

Dimensions: 245 x 260 x 90 mm³