

OPERATING INSTRUCTIONS AND SYSTEM DESCRIPTION FOR THE

PDES-DXH

PNEUMATIC DRUG EJECTION SYSTEM



VERSION 1.6
npi 2020

Table of Contents

1. Safety Regulations	3
2. PDES-DXH System.....	4
2.1. System Description.....	4
2.2. Description of the Front Panel.....	5
2.3. Description of the Rear Panel.....	7
2.4. External Valve	9
Remote Valve Plate	9
microJECT micro Valve Pipette Holder.....	9
2.5. Connections and Operation	10
3. Literature	13
4. Technical Data.....	14

1. Safety Regulations

VERY IMPORTANT: Instruments and components supplied by npí 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. npí electronic disclaims any warranties for such purpose. Equipment supplied by npí 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 npí 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 npí 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.

HIGH PRESSURE PRECAUTIONS: While working with PDES systems and components, always adhere to the appropriate safety measures for handling high pressure pneumatic systems. Before using the PDES or any external pneumatic device please read the manual and instructions carefully.

- Always turn off pressure source before connecting or disconnecting the PDES system.
- Any component used (tubes, fittings etc.) must be specified for the maximum possible pressure.
- Do not use any aggressive, combustible or explosive gases. Only gases such as air, nitrogen, carbon dioxide, helium, etc. are allowed.
- Use only dry, oil free, filtered gases (max. 5 µm filter is allowed).
- Maximum input pressure must be 5 bar (1 bar = 100,000 Pa).

2. PDES-DXH System

2.1. System Description

Pressure ejection is a convenient method for applying both ionic and non-ionic solutions from micropipettes. This method is popular for two reasons. First, it can apply to almost all solutions. Second, the amount of solution ejected can be monitored by viewing the tip of the micropipette under a microscope.

The PDES-DXH is designed for pressure ejection of drugs in physiological and pharmacological studies. PDES systems are equipped with pressure outputs ($P_{\max} = 4 \text{ bar} / 58 \text{ psi}$ or $2 \text{ bar} / 29 \text{ psi}$) for drug application with fine-tipped micropipettes or for controlled droplet application from large-tipped pipettes. Digital or analog timer and a remote control unit facilitate the use of these instruments.

In order to speed up drug ejection, PDES-DXH system are available with the *microJect* option. These systems are equipped with a micro valve located in an injection holder. The micro valve has a switch time of less than 1 ms and a very small volume that has to be controlled, resulting in application times in the ms range.

The PDES-DXH is the standard system. Each system is housed in a 19" cabinet that can be mounted in a rack. It comprises of one ejection channel and a HOLD channel. Each channel consists of a precision pressure regulator, a digital pressure display, electronically controlled valves, control electronics and a regulated power supply. Ejection of drugs can be activated manually or via a TTL input. The operational status is indicated by a red/green LED. With the HOLD channel a low "retain" pressure can be applied (analogous to the "retaining" current used in iontophoretic systems) or the system can be exhausted.

Mode of operation and timing is selected by controls at the front panel. If the internal timers are used, the eject time is preset by a control and the time base can be selected by a toggle switch. The operational status is indicated by a red/green LED. Red means that the channel has been turned on and the valve is closed, green indicates that the valve is open and injection takes place.

The remote control unit (optional) is housed in a small box. For each channel it has a red LED that indicates if the respective channel is turned on and a toggle switch with a temporary and a fix position for starting drug application.

The pressure connectors (quick connect type) are located at the rear panel of the instrument. Each channel is equipped with an INPUT coupling where the pressure source is connected, an OUTPUT coupling where the injection pipette is connected and an HOLD coupling where a low "hold" pressure can be applied in order to avoid capillary effects at the tip of the injection pipette or to apply a "retain" negative pressure (analogous to the "retaining" current used in iontophoretic systems). Please contact npi electronic for details.

The injection pressure is preset by a precision pressure regulator. The pressure is displayed on a digital manometer. Additional pneumatic devices (tubes, fittings connectors, filters etc.) are available. Please contact npi electronic for details.

A calibration procedure allowing quantitative drug application is described by Hofmeier and Lux (1981) (see chapter 3).

2.2. Description of the Front Panel

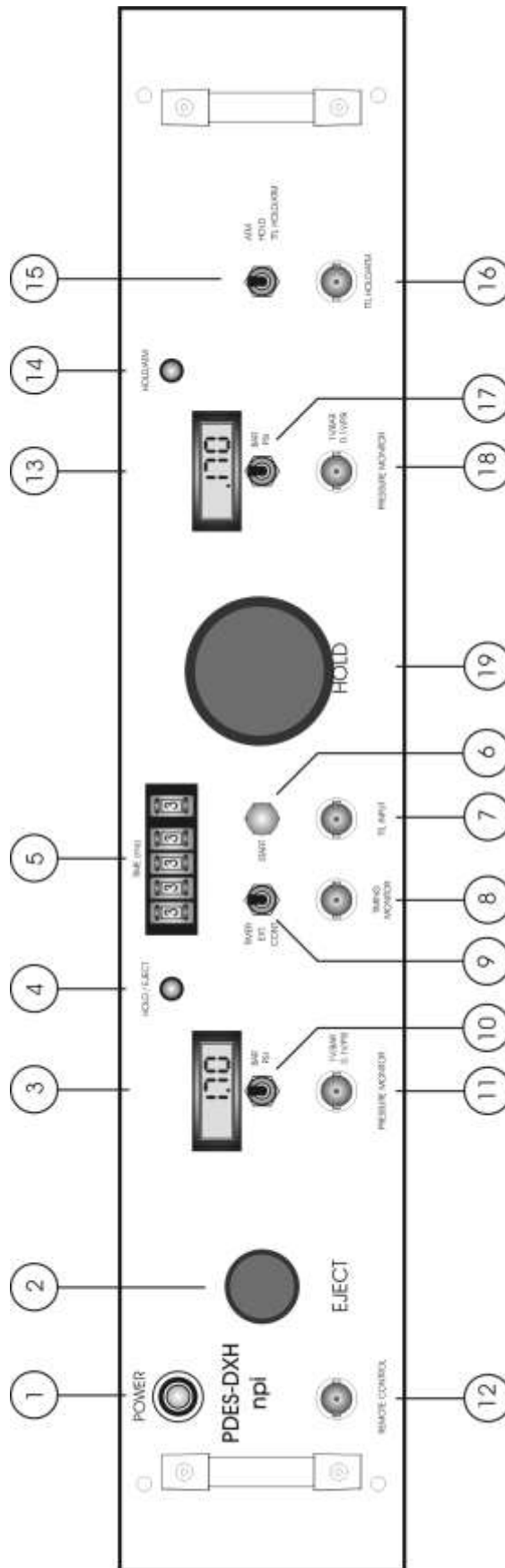


Figure 1: PDES-DXH front panel view (the numbers are related to those in the text below)

In the following description of the front panel elements, each element has a number that is related to that in Figure 1. The number is followed by the name (in uppercase letters) written on the front panel and the type of the element (in lowercase letters). Then, a short description of the element is given.

(1) POWER push button

Push button to turn POWER on or off

(2) Pressure regulator for the EJECT pressure

Rotary knob for regulating the EJECT pressure

(3) PRESSURE MONITOR

Digital display for the EJECT pressure (X.XX bar or XXX.X psi)

(4) HOLD / EJECT LED

Indicates the operational status of the EJECTION pressure

red light: valve is in HOLD/VACUUM position

green light: valve is in EJECT position

(5) TIME (ms) tumble-wheel switch

Five digit tumble-wheel switch to set the ejection time (XXXX.X ms) in TIMER mode

(6) START button

Push button to start operation. The START button can be used to start ejection in TIMER or EXT. mode.

In TIMER mode pressing the START button opens the valve for the time set by #5.

In EXT. mode pressing the START button opens the valve as long as the button is pressed

(7) TTL INPUT connector

BNC connector for external operation

In TIMER mode a TTL HI (+5V) signal opens the valve for the time set by #5.

In EXT. mode a TTL HI (+5V) signal opens the valve as long as the signal level is HI

(8) TIMING MONITOR connector

BNC connector providing a TTL signal that indicates the status of the regulated injection valve (HI = valve open, LO = valve closed)

(9) TIMER, EXT., CONT. switch

Switch to set the operation mode (see also chapter (8))

(10) BAR / PSI switch

Switch to select the unit of the reading of the display (#3) and the output at PRESSURE MONITOR (#11)

(11) PRESSURE MONITOR connector

BNC connector monitoring the pressure output, sensitivity: 1 V / bar or 0.1 V / psi, respectively

(12) REMOTE CONTROL connector

Connector for the REMOTE CONTROL button for the EJECT channel

(13) PRESSURE MONITOR

Digital display for the HOLD pressure (X.XXX bar or XX.XX psi).

Note: A HOLD pressure of less than 11 mbar cannot be displayed!

(14) HOLD / ATM LED

Indicates the source of the HOLD pressure

red light: pipette has ATM pressure (see also chapter 2.3)

green light: pipette has HOLD pressure

(15) ATM / HOLD / TTL HOLD/ATM switch

Switch to set the HOLD pressure

ATM: the pressure is ATM (see also chapter 2.3)

HOLD: the pressure is HOLD (set by #19)

TTL HOLD/ATM: the pressure is set by a TTL signal at connector #16. TTL HI = pressure is HOLD; TTL LOW = pressure is ATM

(16) TTL HOLD/ATM connector

BNC for a TTL signal to set the source of the HOLD pressure

TTL HI = pressure is HOLD

TTL LOW = pressure is ATM

(17) BAR / PSI switch

Switch to select the unit of the reading of the display (#12) and the output at PRESSURE MONITOR (#18)

(18) PRESSURE MONITOR connector

BNC connector monitoring the HOLD pressure output, sensitivity: 10 V / bar or 1 V / psi, respectively

(19) Pressure regulator for the HOLD pressure

Rotary knob for regulating the HOLD pressure

2.3. Description of the Rear Panel

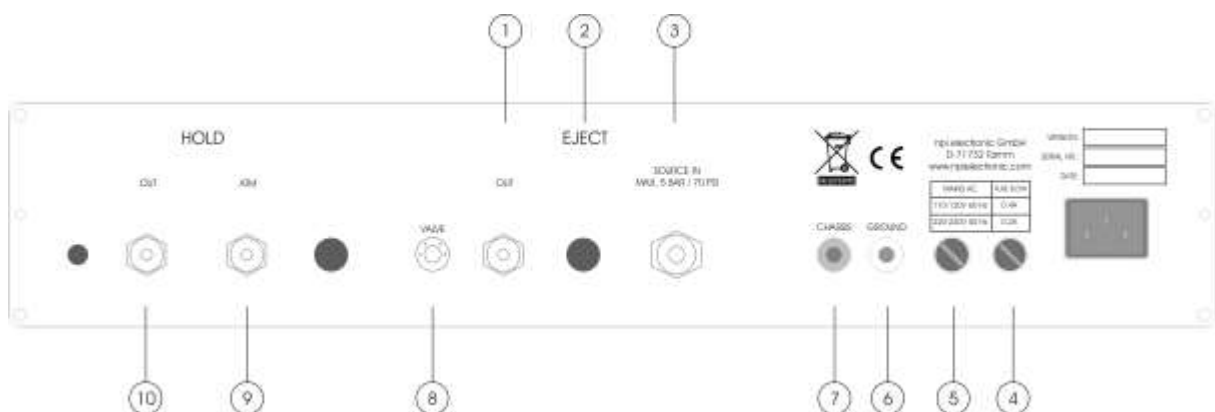


Figure 2: PDES-DXH rear panel view (the numbers are related to those in the text below)

The following connectors are located at the rear panel.

EJECT section

(1) OUT connector

Connector for connecting the ejection tubing of the external valve (black). The EJECT pressure is provided at this connector.

(2) EJECT connector

Not installed. It is replaced by the OUT connector.

(3) SOURCE IN connector

Connector for connecting the filtered, dry and oil-free gas source for EJECT pressure (5 bar max.).

Note: npi recommends an input pressure of 1 bar more than the maximum output pressure.

(4) FUSE

Unplug the instrument when replacing the fuse or changing line voltage. Turn knob to open. Replace fuse only with an appropriate specified type (see chapter 4)

(5) Voltage SELECTOR

Rotary switch for selecting the operating voltage (115 V / 230 V).

Caution: Always switch to the appropriate voltage before connecting the PDES to power.

(6) GROUND

This connector is linked to the internal system ground which has no connection to the 19" cabinet (CHASSIS) and the mains ground to avoid ground loops.

(7) CHASSIS

This connector is linked to mains ground (green / yellow wire, protective earth).

(8) VALVE

Connector for the electrical connection of the external VALVE located on a remote valve plate.

HOLD section

(9) ATM

Usually this connector is left open providing atmospheric pressure.

But another pressure/vacuum source can be connected. In this case, the pressure or vacuum is applied to the pipette if switch #15, Figure 1 is set to ATM or if ATM is selected by a TTL signal.

(10) OUT

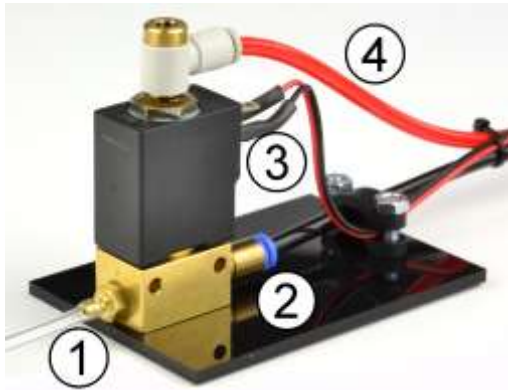
Connector for connecting the hold tubing of the external valve (red). The HOLD pressure is provided at this connector.

2.4. External Valve

External valve(s) for the PDES system are either mounted on a remote valve plate or in the *microJECT* micro valve pipette holder.

Remote Valve Plate

The valve which is controlled by the PDES-DXH is located on the remote valve plate (see Figure 3). Tubing and cable of the external valve are connected at the rear panel of the PDES system.



- 1: connector with tubing to pipette holder
- 2: connector for EJECT pressure (marked with black tubing)
- 3: electrical connection
- 4: connector for HOLD pressure (marked with red tubing)

Figure 3: PDES remote valve

microJECT micro Valve Pipette Holder

The valve which is controlled by the PDES-DXH is located in the micro valve pipette holder, called *microJECT* (see Figure 4). Tubing and cable of the *microJECT* are connected at the rear panel of the PDES system (refer to chapter 2.3).

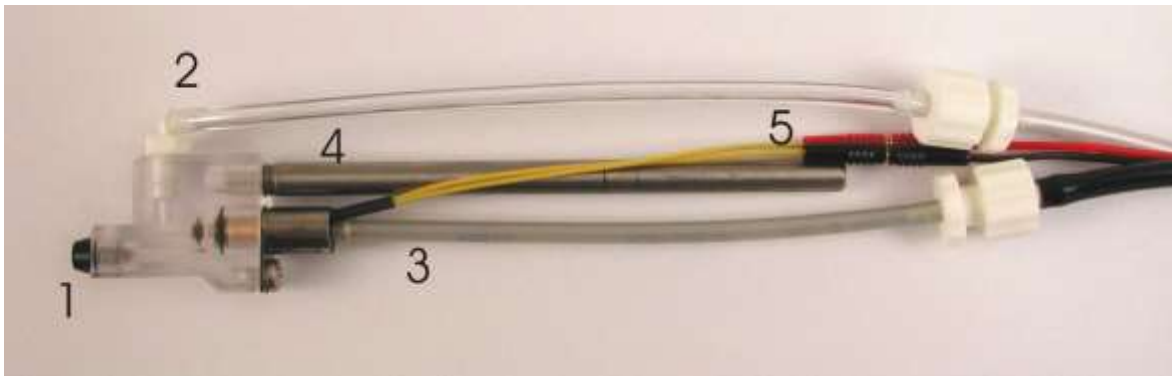


Figure 4: PDES *microJECT*

- 1: pipette connector
- 2: connector for EJECT pressure (marked with black tubing)
- 3: connector for HOLD pressure (marked with red tubing)
- 4: holding bar
- 5: electrical connection

2.5. Connections and Operation

- Connect the gas source to the INPUT connector at the rear panel. The gas source must be clean and filtered by a 5 μm filter element.

Important: Do not connect sources of OXYGEN or COMBUSTIBLE or EXPLOSIVE gases!!

- Connect the red tubing of the external valve (EJECT pressure) to the EJECT OUT connector at the rear panel #1, Figure 2.
- Connect the black tubing of the external valve (HOLD pressure) to the HOLD OUT connector at the rear panel #10, Figure 2.
- Turn on pressure and adjust both, HOLD and EJECTION pressure to the desired values using pressure regulators EJECT and HOLD, respectively.
- Enable or disable HOLD / ATM pressure with switch #12, Figure 1.
- Connect a digital timing unit to the TTL INPUT BNC connector at the front panel (optional).
- For remote operation connect the remote control button (optional) to the REMOTE CONTROL connector.
- Turn channel on (TIMER control).
- The LED is red when no ejection occurs (HOLD pressure at the pipette) and green when the pressure is applied to the pipette.
- Select the operation mode using the respective toggle switch at the front panel (see Figure 1).
 - In the CONT. position the application can be controlled manually, i.e. as long as the CONT. position is set pressure is applied to the pipette.
 - In the EXT. position the duration of the application is determined directly by the duration of the TTL pulse.
 - In the TIMER position the duration is preset TIME SET unit (#5, Figure 1) of the PDES.
- Use the START button to test the system. The START button can be used to start ejection in TIMER or EXT. mode. In TIMER mode pushing the START button starts ejection for the time set by TIME SET unit. In EXT. mode ejection lasts as long as the START button is pushed.

If EJECTION stops, a HOLD pressure remains at the pipette if switch #15, Figure 1 is set to HOLD. If switch #15, Figure 1 is set to ATM, the pipette is passively exhausted (atmospheric pressure).

Note: The PDES always spends some air (3 liters per minute maximum). Therefore, some fizzling may be heard.

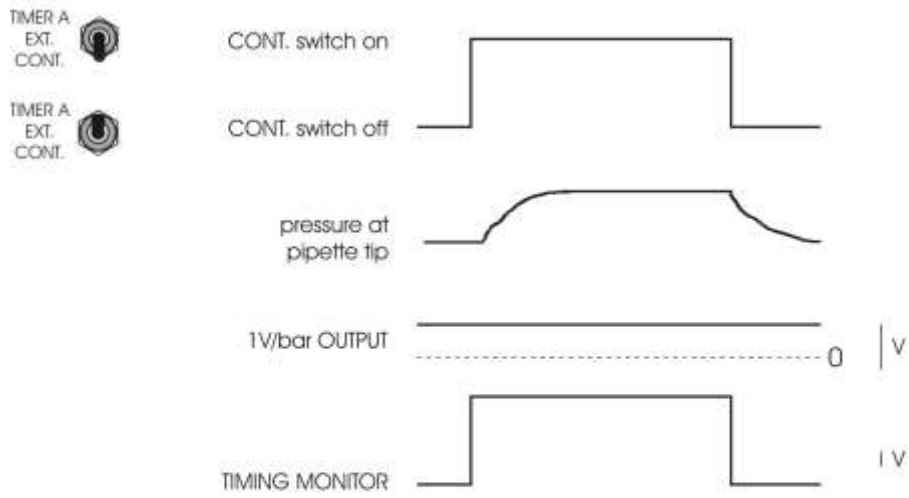
- REMOTE button (option): The selected operation mode (at the controller) determines the way the REMOTE button works.
 - In the CONT. mode the remote control unit is disabled.
 - In the EXTERN mode the valve is open as long the REMOTE button is pressed
 - In the TIMER mode the internal timer is triggered once by pressing the button.



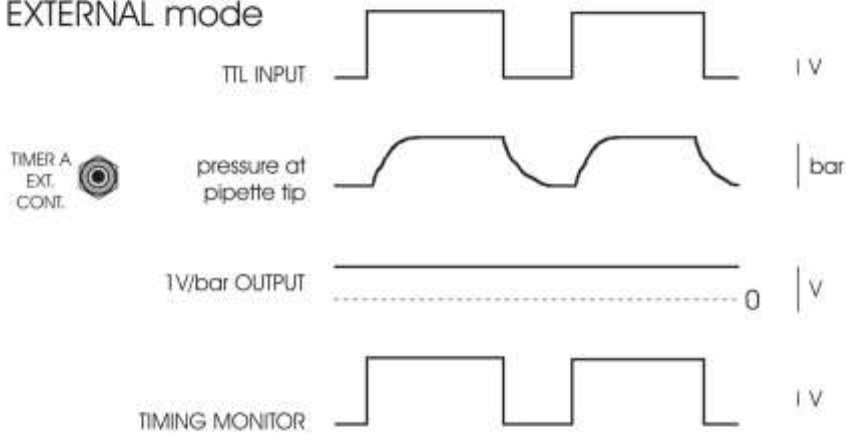
Figure 5: PDES remote button

Note: The remote button is functional on the EJECT channel only.

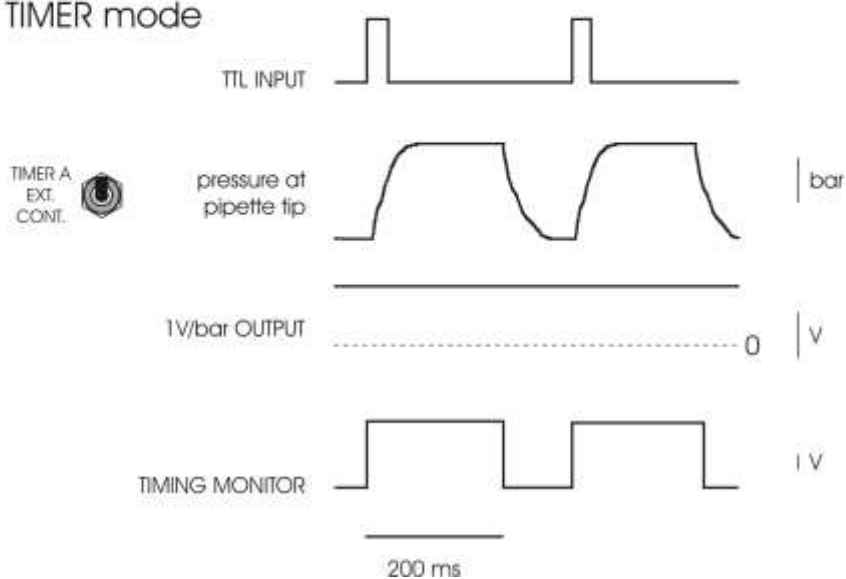
Manual mode



EXTERNAL mode



TIMER mode



If the internal timer is set to 200 ms

Figure 6: operation modes of the PDES-DXH

3. Literature

- Bickmeyer, U., Heine, M., Manzke, T., & Richter, D. W. (2002). Differential modulation of Ih by 5-HT receptors in mouse CA1 hippocampal neurons. *Eur.J.Neurosci.* **16**, 209-218.
- Guzman, J. N., Hernandez, A., Galarraga, E., Tapia, D., Laville, A., Vergara, R., Aceves, J., & Bargas, J. (2003). Dopaminergic modulation of axon collaterals interconnecting spiny neurons of the rat striatum. *J Neurosci.* **23**, 8931-8940.
- Hahnel, C. (1992). Quantitative Microinjection, in: H. Kettenmann and R. Grantyn (eds.) *Practical Electrophysiological methods*, Wiley-Liss, New York.
- Hanganu, I. L. & Luhmann, H. J. (2004). Functional Nicotinic Acetylcholine Receptors on Subplate Neurons in Neonatal Rat Somatosensory Cortex. *Journal of Neurophysiology* **92**, 189-198.
- Heck, N., Kilb, W., Reiprich, P., Kubota, H., Furukawa, T., Fukuda, A., & Luhmann, H. J. (2006). GABA-A Receptors Regulate Neocortical Neuronal Migration In Vitro and In Vivo. *Cereb Cortex*. doi:10.1093/cercor/bhj135
- Hofmeier, G & Lux, H. D. (1981). The time course of intracellular free calcium and related electrical effects after injection of CaCl₂ into neurons of the snail *Helix pomatia*, *Pflügers Arch.* **391**, 242-251.
- **Lalley, P.M. (1999) Microiontophoresis and Pressure Ejection, in: U. Windhorst, and H. Johansson (eds) Modern Techniques in Neuroscience Research, Springer, Berlin Heidelberg, New York.**
- Misgeld, U. (1992). Droplet application of Agonists, in: H. Kettenmann and R. Grantyn (eds.) *Practical Electrophysiological methods*. Wiley-Liss, New York
- Partridge, L. D., Zeilhofer, H. U. & Swandulla, D. (1998). Combined Whole-Cell and Single-Channel Current Measurement with Quantitative Ca²⁺ Injection or Fura-2 Measurement of Ca²⁺. In P.M. Conn (Ed.) Ion Channels Part B, *Methods in Enzymology* **293**, Academic Press San Diego.
- Ponimaskin, E., Dumuis, A., Gaven, F., Barthet, G., Heine, M., Glebov, K., Richter, D. W., & Oppermann, M. (2005). Palmitoylation of the 5-Hydroxytryptamine_{4a} Receptor Regulates Receptor Phosphorylation, Desensitization, and {beta}-Arrestin-Mediated Endocytosis. *Molecular Pharmacology* **67**, 1434-1443.
- Wetzel C. H., Oles, M., Wellerdieck, C., Kuczkowiak, M., Gisselmann, G. and H. Hatt (1999) Specificity and Sensitivity of a Human Olfactory Receptor Functionally Expressed in Human Embryonic Kidney 293 Cells and Xenopus Laevis Oocytes, *J. Neurosci.* **19**(17):7426–7433

4. Technical Data

Source gas: air, nitrogen, helium etc. no aggressive, combustible or explosive gases, filtered by a 5 µm filter element.

Pressure range:

input pressure: max. 5 bar (1 bar = 10⁵ Pa = 14.5 psi)

eject pressure control range: 20 mbar-2.1 bar, 0.3-30 psi
or
20 mbar-4 bar, 0.3-60 psi

residual air pressure: atmosphere

(if EJECT is set to zero)

hold pressure control range: 11-140 mbar, 0.016-2 psi

Input and output couplings:

quick connect type

Tubing:

input: 6 mm OD; output: 4 mm OD

Status / EJECT time indicator:

red/green LED

red light: channel has been turned on, valve is closed

green light: valve is open or valve not connected

Source HOLD pressure indicator:

red/green LED

red light: pipette has ATM pressure

green light: pipette has HOLD pressure

Mode selection:

three position toggle switch, (TIMER, CONT., EXT.)

Typical minimum valve switch time:

internal valve:	~ 100 ms
standard external valve:	~ 20 ms
EHM MicroJECT:	< 5 ms

Digital timer:

100 µs – 9.9999 s set by five digit tumble-wheel switch (0000.0-9999.9)

Pulse input:

standard BNC connector, TTL (LO = 0-2.5 V, HI = 2.5-12 V), input impedance 10 kΩ

Pulse output:

TIMING MONITOR: standard BNC connector, TTL (LO = +0-1 V, HI = +5 V), output impedance 249 Ω

Dimensions:

19" rackmount cabinet, 19" (483 mm), 10" (250 mm), 3.5" (88 mm)

Power requirements:

115/230 V AC, 20-40 W, Fuse: 0.2A / 230V; 0.4 A / 115V