

# OPERATING INSTRUCTIONS AND SYSTEM DESCRIPTION FOR THE

## PDES-02DX

# PNEUMATIC DRUG EJECTION SYSTEM

(with analog timers and digital manometers - PDES-02T-LA)



VERSION 2.1  
npi 2015

npi electronic GmbH, Bauhofring 16, D-71732 Tamm, Germany  
Phone +49 (0)7141-9730230; Fax: +49 (0)7141-9730240  
support@npielectronic.com; <http://www.npielectronic.com>

## Table of Contents

1.	Safety Regulations .....	3
2.	PDES-2DX System .....	4
2.1.	System Description .....	4
2.2.	PDES Components .....	5
2.3.	Description of the Front Panel .....	5
2.4.	Description of the Rear Panel .....	8
3.	External Valves .....	9
	Remote Valve Plate .....	9
	microJECT micro Valve Pipette Holder.....	10
4.	Connections and Operation .....	11
5.	Literature .....	14
	Microinjection into Oocytes .....	15
	Fast Drug Application with microJECT.....	15
6.	Technical Data.....	16

## 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).
- Input pressure must be max. 5 bar (72 psi).

## 2. PDES-2DX 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-2DX is designed for pressure ejection of drugs in physiological and pharmacological studies. PDES systems are equipped with high pressure outputs ( $P_{\max} = 4 \text{ bar} / 58 \text{ psi}$ ) for drug application with fine-tipped micropipettes, but can also be operated in low-range pressure for controlled droplet application from large-tipped pipettes. Digital timers and a remote control unit facilitate the use of these instruments.

In order to speed up drug ejection, PDES-2DX systems 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 pressure is restricted to 2 bar / 29 psi.

The PDES-2DX is the standard system. Each system is housed in a 19" cabinet that can be mounted in a rack. It comprises of two channels. Two systems can be combined to form a 4 channel system. Each channel consists of a precision pressure regulator, an analog 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. Each channel is equipped with an "HOLD" coupling whereby a low "retain" pressure can be applied (analogous to the "retaining" current used in iontophoretic systems).

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. Each channel can be turned off by using a switch that is combined with the timing control. 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 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). The "retain" pressure is generated internally and preset by a precision pressure regulator. Please contact npj 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 npj electronic for details.

A calibration procedure allowing quantitative drug application is described by Hofmeier and Lux (1981) (see chapter 5).

## **2.2. PDES Components**

The following items are shipped with a PDES system:

- ✓ PDES pressure controller
- ✓ Power cord or external power supply
- ✓ User manual

Optional accessories:

- ⇒ Ejection pipette holder with holding bar and LUER connector
- ⇒ Remote control
- ⇒ Tubing for connection the controller to the pressure source
- ⇒ Tubing for connection the ejection pipette holder to the controller
- ⇒ remote valve on a plate for faster application including connection to the controller
- ⇒ *microJECT* pipette holder for ultra-fast application including connection to the controller

## **2.3. Description of the Front Panel**

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. PDES systems are available with one or two channels. Since both channels are identical only channel A is described.

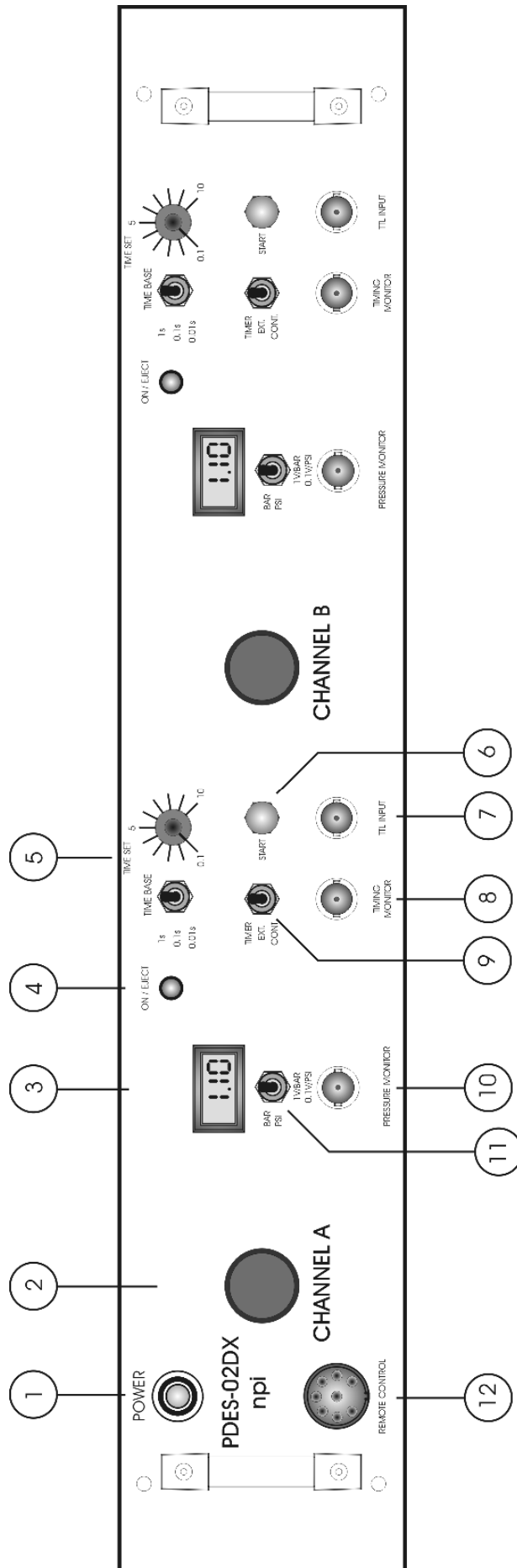


Figure 1: PDES-2DX front panel view (the numbers are related to those in the text below)

**(1) POWER switch**

Switch to turn POWER on or off

**(2) Pressure regulator**

Rotary knob for regulating the pressure of channel A

**(3) Manometer**

Digital display indicating pressure at channel A (X.XX bar or XX.X psi)

**(4) ON / EJECT LED**

Indicates the operational status of channel A.

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

green light: valve is open

**(5) TIME SET switch and timer potentiometer**

Switch to set the TIME BASE of channel A and potentiometer to set the ejection time of channel A. The total ejection time is the reading of this potentiometer multiplied by the reading of the TIME SET switch

**Note:** Due to tolerances of resistors and capacitors the time preset with the analog timer may vary up to 20%!

**(6) START button**

Push button for starting operation of channel A in TIMER or EXT. mode

**(7) TTL INPUT connector**

BNC connector for external operation of channel A with a TTL HI signal (+5 V). In TIMER mode the internal timer is triggered by the TTL signal. In EXT. mode the valve is open as long as the TTL signal is HI

**(8) TIMING MONITOR**

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

**(9) Operation mode switch**

Switch to set the operation mode for channel A. TIMER, EXTERNAL or CONTINUOUS (see also chapter 0).

**(10) PRESSURE MONITOR connector**

BNC connector monitoring the applied pressure. The scaling is dependent on the setting of switch (11), 1V / BAR or 0.1 V / PSI.

**(11) BAR / PSI switch**

Switch to select the unit of the reading of the display (#3) and the output at PRESSURE MONITOR (#10)

**(12) REMOTE CONTROL connector**

Connector for the REMOTE CONTROL unit (see Figure 5)

## 2.4. Description of the Rear Panel

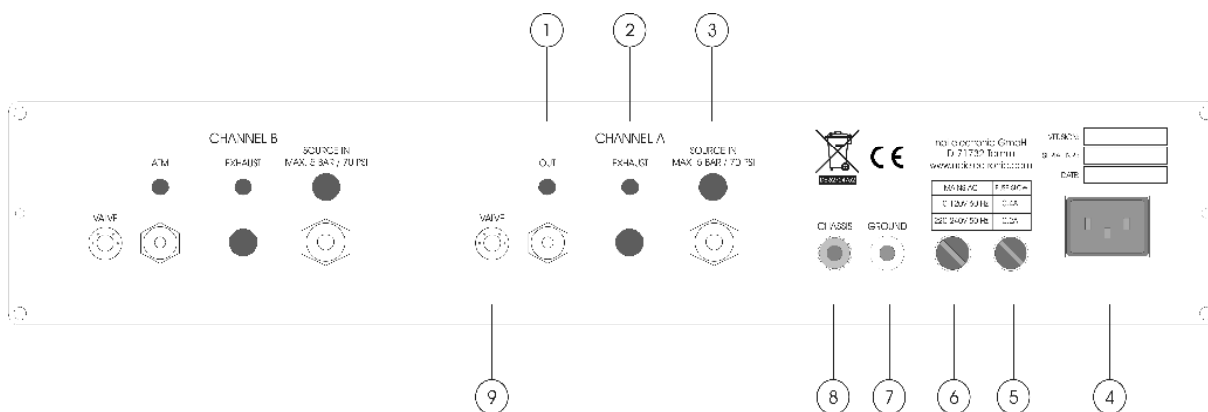


Figure 2: PDES rear panel view (the numbers are related to those in the text below)

The following connectors are located at the rear panel. The connectors are mentioned only for CHANNEL A. Since both channels are identical, only one channel is described.

### (1) OUT connector

Connector for connecting the injection micropipette or for connecting the pressure tubing of the external valve on the remote valve plate. The EJECT pressure is supplied to this connector.

### (2) EXHAUST connector (optional)

Connector for connecting the HOLD pressure source. If no HOLD pressure is connected, the system is exhausted as long as no pressure is applied to the pipette.

### (3) SOURCE IN connector

Connector for connecting the filtered, dry and oil-free gas source (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 6)

### (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 (optional)

Connector for the electrical connection of the VALVE located in a remote valve box or in the *microJECT*.

**Important:** This connector is **not** used for systems without a remote valve or *microJECT*.



### 3. External Valves

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 valves which are controlled by the PDES-02DX are located on the remote valve plate (see Figure 3). Tubing and cable of the external valves are connected at the rear panel of the PDES system (refer to chapter 2.4). Exhaust is usually not connected.

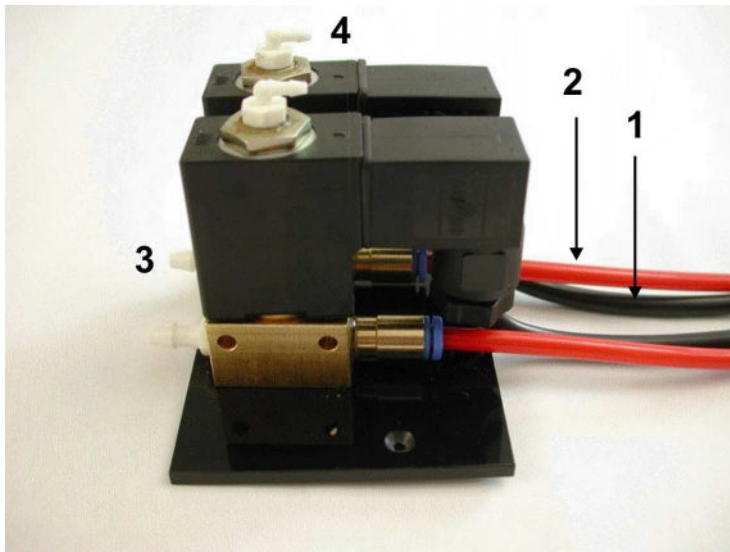


Figure 3: external valves on remote valve plate

- 1: electrical connection (black)
- 2: EJECT pressure tubing (red)
- 3: connector for ejection pipette
- 4: exhaust connector (optional)

*microJECT micro Valve Pipette Holder*

The valve which is controlled by the PDES-02DX 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 2.4). Exhaust is usually not connected.

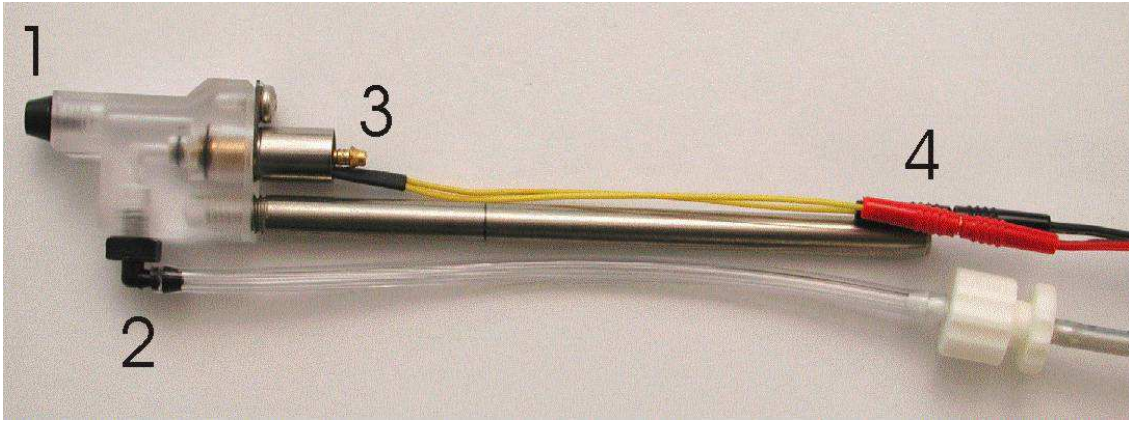
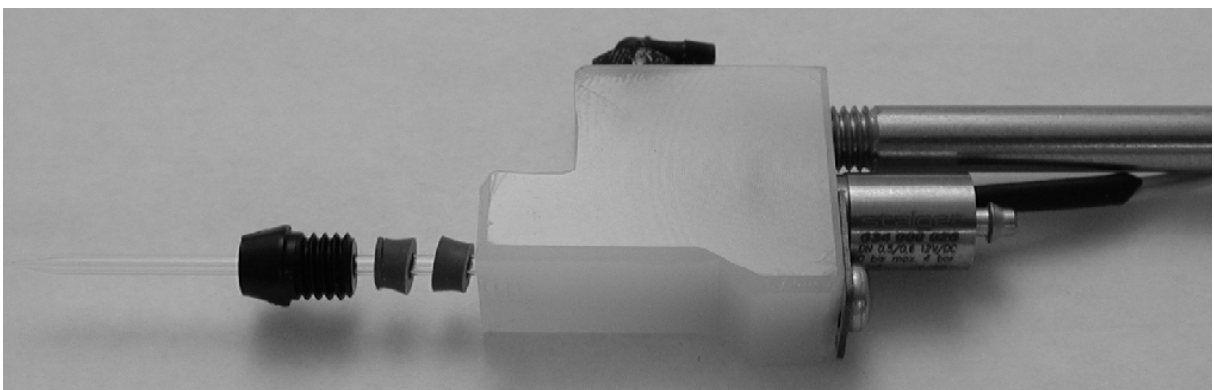


Figure 4: *microJECT*

- 1: pipette connector
- 2: connector for EJECT pressure
- 3: connector for EXHAUST pressure (optional)
- 4: electrical connection

**Important:** In experiments with high pressures (> 1 bar) it might be necessary to insert 2 seals in the pipette connector in order to prevent the pipette from moving in any direction.



**Caution:** Pipettes that are not rigidly fixed in the pipette connector might be pressed out of the connector by high pressure. Always adhere to the appropriate safety measures.

## 4. Connections and Operation

- ❑ Connect the gas source to the INPUT connector at the rear panel.

***Important:*** Do not connect sources of OXYGEN or COMBUSTIBLE gases!! The gas source MUST be dry and oil free!! Please use a degreaser and filter the gas source by a 5 µm filter element.

- ❑ Connect the pressure tubing of each external valve to the respective OUT connector, and the electrical connector to the respective VALVE connector at the rear panel.
- ❑ Connect your ejection pipette(s) to the external valve(s).
- ❑ Turn on pressure and adjust the EJECTION pressure to the desired value using the pressure regulator.
- ❑ Connect a digital timing unit to the TTL INPUT BNC connector at the front panel (for EXT. operation).
- ❑ For remote operation connect the remote control unit (option) to the REMOTE CONTROL connector.
- ❑ 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 by the timer control and time base 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 the timer control (#5, Figure 1). In EXT. mode ejection lasts as long as the START button is pushed.
- ❑ The LED is red when no ejection occurs and green when the pressure is applied to the pipette.

***Very important notice:*** Even if an eject channel is not activated, i.e. the valve is closed, there is a pressure at the pipette. Depending on the pressure regulator that is used, the pressure at the pipette with valve closed is between 10 mbar and 100 mbar. If this is unacceptable for your application, the PDES system can optionally be equipped with an additional valve for exhausting. Please contact npj electronic for details.

- ❑ REMOTE CONTROL (option): The selected operation mode (at the controller) determines the way the REMOTE CONTROL works.
  - In the CONT. mode the remote control unit is disabled.
  - In the EXTERN mode the valve is open as long the REMOTE control switch is activated (CONT./TIMED = continuous, START = temporarily).
  - In the TIMER mode the internal timer is triggered once by activation of the REMOTE control switch (CONT./TIMED or START). The green LED indicates which channel has been turned on.



Figure 5: PDES remote control (optional)

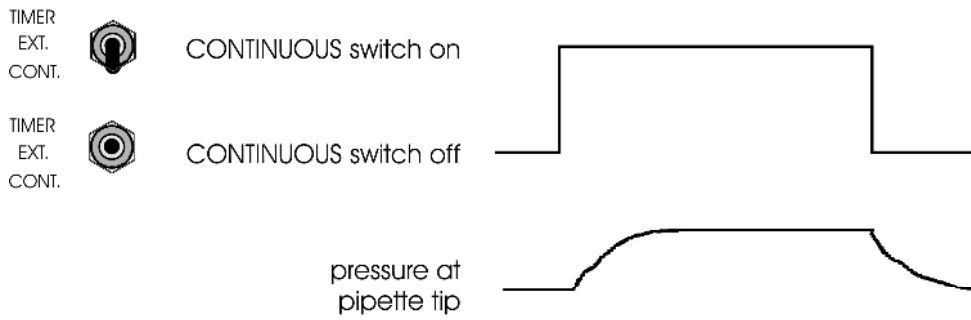
**Note:** Green LED indicates that the valve is not connected properly or continuous operation.

- ❑ REMOTE CONTROL with foot switch (option, see Figure 6): The selected operation mode (at the controller) determines the way the REMOTE CONTROL footswitch works.
  - In the CONT. mode the remote control unit is disabled.
  - In the EXTERN mode the valve is open as long the REMOTE control foot switch is activated.
  - In the TIMER mode the internal timer is triggered once by activation of the REMOTE control foot switch

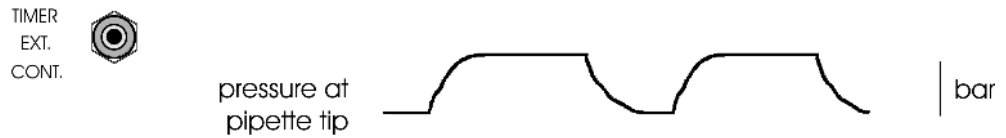


Figure 6: PDES remote control footswitch as 2-channel or single channel version.

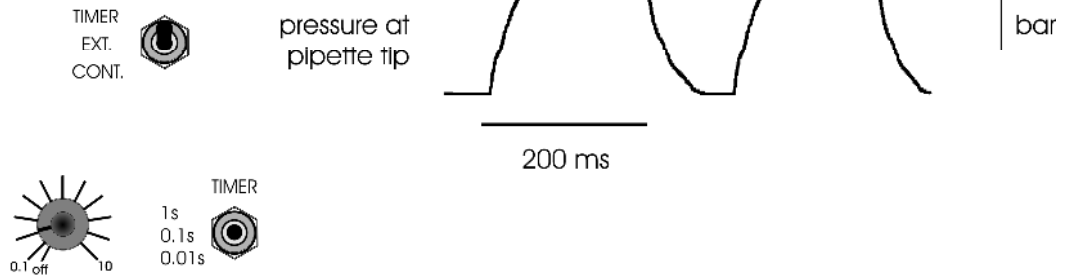
## Manual mode



## EXTERNAL mode



## TIMER mode



if the internal timer  
is set to 200 ms

Figure 7: operation modes of the PDES

## 5. 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.
- ❑ Eggermann, E. & Feldmeyer, D. (2009). Cholinergic filtering in the recurrent excitatory microcircuit of cortical layer 4. *Proc.Natl.Acad.Sci.U.S.A.* **106**, 11753-11758.
- ❑ Garaschuk, O., Milos, R. I., & Konnerth, A. (2006). Targeted bulk-loading of fluorescent indicators for two-photon brain imaging in vivo. *Nat.Protocols* **1**, 380-386.
- ❑ Guzman, J. N., Hernandez, A., Galarraga, E., Tapia, D., Laville, A., Vergara, R., Aceves, J., &argas, 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.
- ❑ Hofmeier, G & Lux, H. D. (1981). The time course of intracellular free calcium and related electrical effects after injection of CaCl<sub>2</sub> into neurons of the snail *Helix pomatia*, *Pflügers Arch.* **391**, 242-251.
- ❑ Kolbaev, S. & Draguhn, A. (2008). Glutamine-induced membrane currents in cultured rat hippocampal neurons. *Eur.J Neurosci.* **28**, 535-545.
- ❑ **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.**
- ❑ Langer, J. & Rose, C. R. (2009). Synaptically induced sodium signals in hippocampal astrocytes in situ. *J Physiol.* **587**, 5859-5877.
- ❑ Lin, B. J., Chen, T. W., & Schild, D. (2007). Cell type-specific relationships between spiking and [Ca<sup>2+</sup>]<sub>i</sub> in neurons of the Xenopus tadpole olfactory bulb. *Journal of Physiology* **582**, 163-175.
- ❑ Marandi, N., Konnerth, A., & Garaschuk, O. (2002). Two-photon chloride imaging in neurons of brain slices. *Pflugers Arch.* **445**, 357-365.
- ❑ 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 Ca<sup>2+</sup> Injection or Fura-2 Measurement of Ca<sup>2+</sup>. 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<sub>4a</sub> Receptor Regulates Receptor Phosphorylation, Desensitization, and {beta}-Arrestin-Mediated Endocytosis. *Molecular Pharmacology* **67**, 1434-1443.
- ❑ Qiu, J., Yao, S., Hindmarch, C., Antunes, V., Paton, J., & Murphy, D. (2007). Transcription factor expression in the hypothalamo-neurohypophyseal system of the dehydrated rat: upregulation of gonadotrophin inducible transcription factor 1 mRNA is mediated by cAMP-dependent protein kinase A. *J Neurosci.* **27**, 2196-2203.

- ❑ Stepanyan, R. & Frolenkov, G. I. (2009). Fast adaptation and Ca<sup>2+</sup> sensitivity of the mechanotransducer require myosin-XVa in inner but not outer cochlear hair cells. *Journal of Neuroscience* **29**, 4023-4034.
- ❑ Wolfram, V. & Juusola, M. (2004). The Impact of Rearing Conditions and Short-Term Light Exposure on Signaling Performance in *Drosophila* Photoreceptors. *Journal of Neurophysiology* **92**, 1918-1927.

#### *Microinjection into Oocytes*

- ❑ Wetzel, C. H., Oles, M., Wellerdieck, C., Kuczkowiak, M., Gisselmann, G., & Hatt, H. (1999). Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and *Xenopus Laevis* oocytes. *J.Neurosci.* **19**, 7426-7433.

#### *Fast Drug Application with microJECT*

- ❑ Courjaret, R., Troger, M., & Deitmer, J. W. (2009). Suppression of GABA input by A1 adenosine receptor activation in rat cerebellar granule cells. *Neuroscience*. **162**, 946-958.
- ❑ Heck, N., Kilb, W., Reiprich, P., Kubota, H., Furukawa, T., Fukuda, A., & Luhmann, H. J. (2007). GABA-A receptors regulate neocortical neuronal migration in vitro and in vivo. *Cereb Cortex*. **17**, 138-148.

## 6. Technical Data

### Source gas:

air, nitrogen, helium etc. no aggressive, combustible or explosive gases, oil-free and filtered by a 5 µm filter element.

### Pressure range:

input pressure: max. 5 bar (1 bar =  $10^5$  Pa = 14.5 psi)  
eject pressure control range: 0-4 bar, 0-60 psi  
hold pressure control range: 0-0.7 bar, 0-10 psi (optional)

### 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: EJECT valve is closed  
green light: EJECT valve is open

### 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

Analog timer (standard version): 0.01 - 0.1 s, 0.1 - 1 s and 1 s-10 s with linear control, range set by toggle switch time base selector (0.01 s, 0.1 s or 1 s)

### Pulse input:

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

### Pulse output:

PRESSURE MONITOR: standard BNC connector, sensitivity: 1 V / bar or 0.1 V / psi, output impedance 249 Ω

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

### Remote control (option):

Manual: Toggle switch and status LED for each channel, size 2.5x4x10 cm

Footswitch: size: 6,5 x 9 x 2.5 cm (one channel), 20 x 14 x 4 cm (two channels)