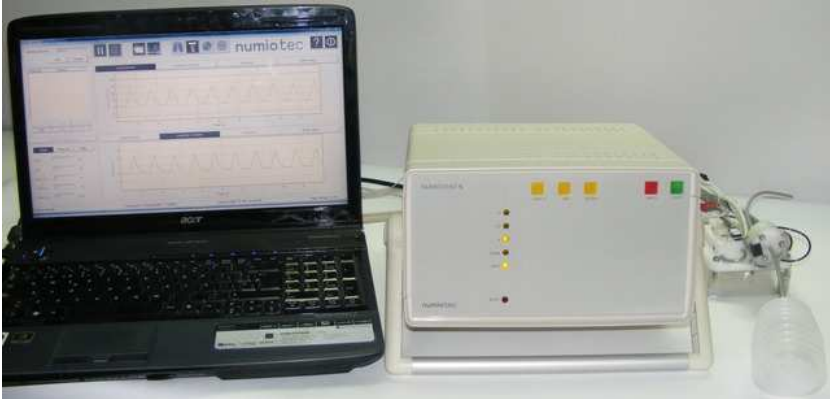


numioV_MRI









**RESPIRATOR FOR SMALL ANIMAL APPLICATIONS WITH
SPECIAL GASES
SYNCHRONIZATION SYSTEM FOR IMAGE ACQUISITION**

USER'S GUIDE

Please read the instruction manual before use.

1. SYMBOLS

 NOTE	Attention: Indicates information that user should pay special attention to.
 CAUTION	Indicates correct operating or maintenance procedures in order to prevent damage to or destruction of the equipment or other property.
 WARNING	Calls attention to a potential danger that requires correct procedures or practices in order to prevent personal injury.
 DANGER	Urgent safety information for hazards that will cause serious injury or death
	Consult operating instructions.
	Disposal of Electrical & Electronic Equipment (WEEE): This product should be handed over to a suitable collection point for the recycling of electrical and electronic equipment. For more detailed information about the recycling of this product, please contact your local city council, household waste disposal service or the retail store where you purchased this product.

2. IMPORTANT SAFEGUARDS



DANGER: To reduce the risk of electrocution:

- Always unplug this product immediately after using.
- Do not use this product close to a water source.
- Do not place or store this product where it can fall or be pulled into a tube or sink.
- Do not place in or drop into water or other liquid.
- Do not reach for a product that has fallen into water, unplug immediately.
- Do not install, maintain or operate this equipment without reading, understanding and following the instructions included in this manual, or injury or damage may result.



WARNING: To reduce the risk of burns, electrocution, fire or injury to people:

- Use the product only for its intended use as described in this manual. Do not use any attachment not recommended by the manufacturer.
- Never operate this product if a) it has a damaged cord or plug, b) it is not working properly, c) it has been dropped or damaged, d) it has been dropped into water. Return the product to a specified service centre for examination and repair.
- Keep the cord away from heated surfaces.
- Never block the air openings of this product or allow objects to fall or be inserted into the air vent openings or place it on a soft surface such as a bed or couch.



CAUTION: To avoid damage in the respirator and accessories:

- Be careful opening the box (es) where device and its modules were packaged for shipping.
- Be sure that the pneumatic and electronic connections are fixed properly according to the manual before proceeding to switch on the device.
- In case of using the device in rooms with power supply instabilities (higher than 10%), use an external power supply stabilizer system with peaks suppression.

- In case of accidental disconnection of a pneumatic tube or electric cables, turn off the unit immediately and be sure of plugging it in the right position before turning on the unit again.
- Do not change the structure or files into the respirator software folder. In case of accidental error, do not use the unit without contacting technical support or reinstall the software.
- The main pneumatic valve must remain in dry conditions. Any liquid (blood,...) coming from the animal can damage the valve and change the measurements accuracy. Please, use a filter between the valve and the animal in applications with risks of humidity and calibrate the system with the new setup before use it.

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4. PRODUCT DESCRIPTION



- 1- Main Box unit (M.01.0000): It is constituted by the main electronic and pneumatic parts of the ventilator.
- 2- Interconnection pack (I.01.0000): This is formed by the AC and electronic cables as well as the pneumatic tubes used in the ventilator functioning.
- 3- Testing and Calibration unit (C.01.0000): Consists of the items needed to calibrate the ventilator system and test it regularly.
- 4- Main Pneumatic Valve unit (V.01.0000): It is the valve used to commute between the air or gas sent to the animal and the resulting expiration gas from the lung of the animal.
- 5- Gas Box unit (G.03.0000), (Optional): It is a pressurized box containing the bag that storage the hyperpolarized gas in a short time before send it to the animal using an air pushing system.
- 6- Automatic Refilling unit (R.04.0000), (Optional): It is used to commute between the Nitrogen or Vacuum and the hyperpolarized gas sent to the Gas Box unit in an automatic way (not shown).
- 7- Recycling unit (E.05.0000), (Optional): It is composed by a pneumatic valve and a storage bag. This unit is used for recycle the hyperpolarized gas expired by the animal in an automatic way (not shown).

5. TECHNICAL SPECIFICATIONS

Pneumatic specifications

Parameters	Min.	Typ.	Max.	Units
Driving gas input pressure	2	3	6	b
Nitrogen input pressure* ¹	0.8	1	1.2	b
Vacuum input pressure* ¹	0.8	1	1.2	b
Primary gas input pressure	0.2	1	1.2	b
Secondary gas input pressure	0.9	1	1.1	b
Hyperpolarized gas input pressure* ¹	0.5	2	4	b
Internal 1b source accuracy	0.9	1	1.1	b
Respiratory rate	1	60	200	bpm
Inspiratory volume * ²	0	-	5	ml
Inspiratory volume accuracy	-	5	10	%
Lung volume measurement accuracy	-	5	10	%
Lung pressure	-	-	40	mb
Lung pressure measurement accuracy	-	-	3	%
PEEP range * ³	0	-	20	mb

*¹- For use with hyperpolarized gas.

*²- For a respiratory rate of 60 breaths per minute.

*³- According to the Lung pressure values.

Electrical & Time specifications

Parameters	Min.	Typ.	Max.	Units
220V power supply input voltage	200	220	240	V
Power frequency	50	-	60	Hz
Distance between main pneumatic valve sensors and the main box	0.5	3	5	m
Distance between pressurized box sensors and the main box	0.5	3	5	m
USB power supply	-	5	-	V
Over-pressure protection response time	50	100	150	ms
Synchronism signal	4.7	5	5.3	V
Synchronism output impedance	49.5	50	50.5	Ω

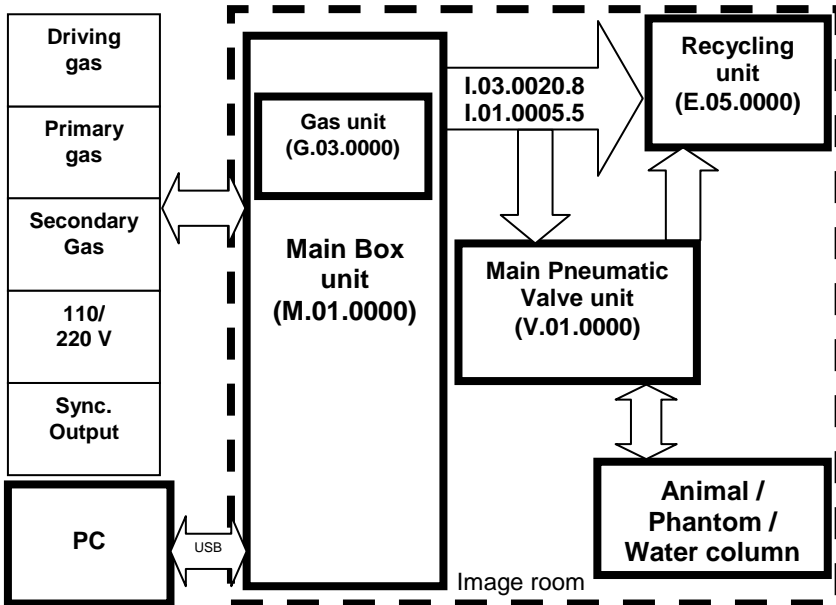
6. STORAGE



CAUTION: In order to preserve the device when it is not in use:

- Leave the unit and modules in place with the adequate temperature and humidity conditions (less than 60% of humidity and room temperature between 10 and 40°C).
- Be careful especially with electric cables to avoid damage when twisting.
- Leave the main pneumatic valves in the original package when they are not in use.
- Do not let pieces or optional modules of the system unattended; protect them as best as possible.
- Keep all accessories dry; avoid direct sunlight.

7. INSTALLATION INSTRUCTIONS



General diagram of the ventilator system

Pneumatic installation

The pneumatic connections of the ventilator were designed for an easy plugging. Additionally, all pneumatic ports are labeled to make the proper plugging easy in case of accidental disconnection. The main box is always supplied with the Interconnection pack.

⚠ CAUTION: Do not turn on the air pressurized source until all pneumatic module and tubes are completely connected.



Rear panel of the device with the pneumatic connection only

A	Input for the driving gas.	D	Input for the secondary gas
B	10 ways pneumatic connector	E	Input of Nitrogen (optional)
C	Output of the internal 1b gas or input for the primary gas	F	Input of vacuum (optional)

Follow the next steps to assemble the pneumatic parts:

- Connect the Driving gas source to the port **A** using a 6 mm external diameter semi-rigid tube (polyurethane). The other tube end must be connected in the external pressure supply point.
- Connect the 10 ways pneumatic connector in the corresponding pneumatic connector **B**. Then, lock the connector using the two screws for it. The tubes are connected to the 10 ways pneumatic connector according the Table below.

Signals description	Position
Inspiration 1 control line to Main Pneumatic Valve	1
Inspiration 2 control line to Main Pneumatic Valve	2
Expiration control line to Main Pneumatic Valve	3
Recycling 1 control line to recycling valve	4
Recycling 2 control line to recycling valve	5


Flow regulated primary gas to Main Pneumatic Valve	6
Flow regulated secondary gas to Main Pneumatic Valve	7
Nitrogen/Vacuum control line to Automatic Refilling unit *	8
Nitrogen/Vacuum line to Automatic Refilling unit *	9
Air control signal to deliver the gas in the Gas Box unit *	10

* For use with hyperpolarized gas, not used in other case.

- To use the Automatic Refilling Module, connect the external Nitrogen source to port **E** by means of a 4 mm external diameter gray semi-rigid tube (polyurethane), and in the same way connect the external source of vacuum to port **F**.
- In case of using an external gas mixer unit or for supplying directly a secondary gas to the animal, for example Oxygen, disconnect the tube between port **C** and **D**, plug the primary gas source to port **C** and plug the secondary gas source to port **D** by mean of a 4mm semi-rigid tube (polyurethane).

Electrical installation

All required electric cables are supplied in the Interconnection pack.

 **DANGER:** In case of loss or damage in cable, please replace it immediately by one similar supplied by the manufacturer.



Picture of the back panel of device with the electrical and pneumatic connections

G	Connector for the Main pneumatic valve & pressurized buffer and Tedlar bag module sensors	I	110 or 220V AC connector
H	Output to synchronize with the image acquisition	J	USB type B connector for controlling the device from an external PC

Follow the next steps for the electrical connection of the unit:

- Connect the round terminal of the sensors cable in the sensors port **G**. The other terminal of the cable must be located in the experiment area, as close as possible to the place where the animal will be ventilated, for example on the bed of the Image device.
- Connect the USB cable type B in the USB port **J**. The other terminal of the cable Type A should be connected to the corresponding port in the manager, for example in the PC.

⚠ NOTE: The only manager supported today for controlling the ventilator is a personal computer running Windows XP or later.

- In case of using the unit with synchronization with imaging devices, connect the synchronism signal cable in the sync out port **H** for triggering the image acquisition (see Acquisition mode operation). The other terminal of the synchronism cable must be connected to the proper connector in the image acquisition device.

⚠ CAUTION: Be sure the connection with the image acquisition device is properly done. Contact the device manufacturer in case of

- doubts. Improper connection of this cable may cause damage in both ventilator and acquisition imaging device.
- Attach the 220V power supply cable corresponding connector I. Plug the other terminal to the electrical line once you have the device ready for operation.

Main pneumatic valve installation or replacement

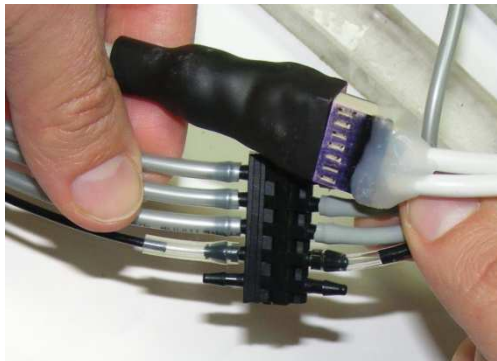
Plugging and unplugging the main pneumatic valve is a common operation according to the experiment requirements and the type of animal to be ventilated.

⚠ NOTE: Each main pneumatic valve is ready to be used in a type of animal. Do not use a valve for a different animal without instructed by the manufacturer.

⚠ WARNING: Main pneumatic valves are supplied with a calibration file. However, it is recommended to perform calibration procedure in the particular lab conditions if high accuracy is required. The main pneumatic valve is placed in the experimental area, as close as possible to the place where the animal will be ventilated, for example on the bed of the MRI device.

Follow the next steps to connect the main pneumatic valve:

- Plug the male electrical connector marked in black of the main pneumatic valve into the female electrical connector marked in black of the interconnection module.
- Plug the black pneumatic connector of the main pneumatic valve to the black connector of the interconnection pack.



Pneumatic connection of the main pneumatic valve

⚠ NOTE: To avoid undesired motion of the main pneumatic valve during the experiments, fix it to the animal bed. An example is shown in the next picture.



Main pneumatic valve fixation in the animal bed

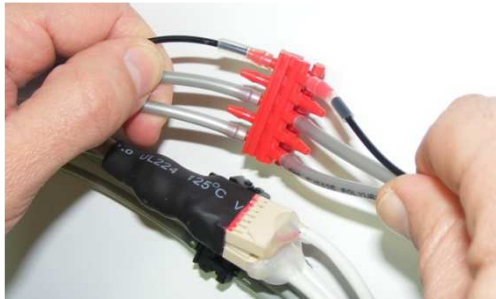
Gas Box unit installation (Optional)

⚠ NOTE: The gas box is ready to be used in all the animal types supported by the ventilator.

⚠ WARNING: The gas box calibration parameters are stored in each main pneumatic valve calibration file. Calibration should be performed at installation. There is no need for the user to calibrate the gas box. The gas box is placed in the experimental area, near of the main pneumatic valve and as close as possible to the place where the animal will be ventilated, for example on the bed of the MRI device.

Follow the next steps to connect the gas box unit:

- Plug the male electrical connector marked in red of the gas box into the female electrical connector marked in red of the interconnection module.
- Plug the red pneumatic connector of the gas box to the red connector of the interconnection pack.



Pneumatic connection of the gas box

- Place the main pneumatic valve above of the gas box, unplug the black pneumatic tube marked as V.01.0000.18B from the main pneumatic valve and plug the pneumatic tube labeled as G.01.0000.5 between the output of the gas box and the main pneumatic valve, as shown in the next picture:



Pneumatic connection between the main pneumatic valve and the gas box

⚠NOTE: To avoid undesired motion of the gas box during the experiments, fix it to the animal bed. An example is shown in the next picture.



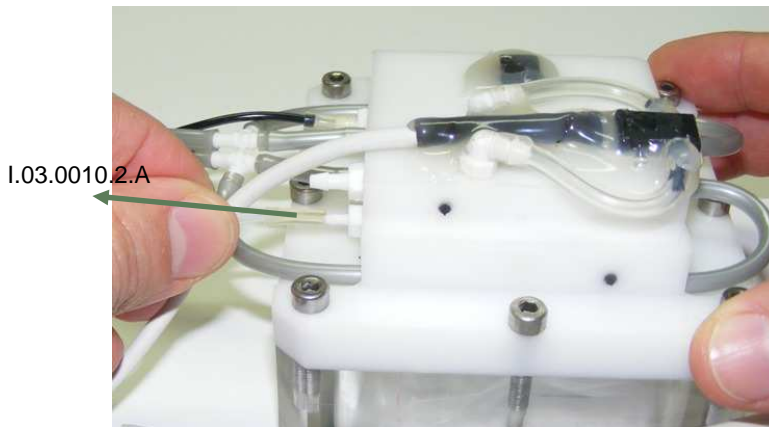
Gas box fixation in the animal bed

Automatic Refilling unit installation (Optional)

⚠NOTE: The Automatic Refilling unit is placed outside the experimental area, as near as possible to the hyperpolarized gas cell and the Main Box unit.

Follow the next steps to connect the Automatic Refilling unit:

- Plug the pneumatic tube marked as I.03.0020.8.9 in the N2/Vac port of the Automatic Refilling unit.
- Plug the pneumatic tube marked as I.03.0020.8.8 in the N2/Vac_Cont port of the Automatic Refilling unit.
- Plug the pneumatic tube marked as I.03.0020.8.7 in the Hyp_Cont port of the Automatic Refilling unit.
- Plug the pneumatic tube marked as I.03.0010.2.A in the Out port of the Automatic Refilling unit and the other end marked as I.03.0010.2.B in the gas_in port of the Gas Box unit.



Gas input connection in the gas box unit

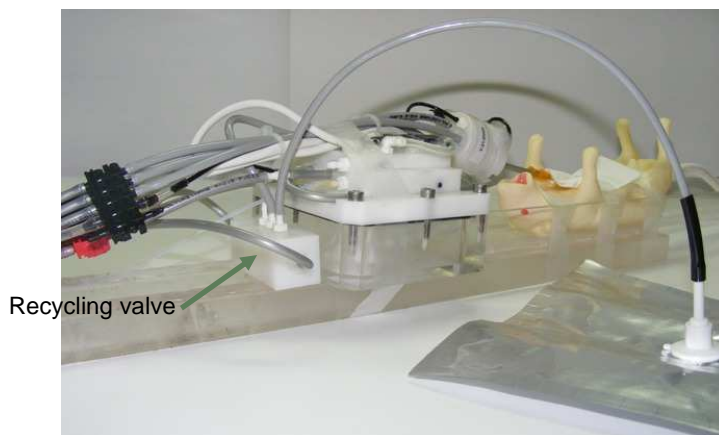
Recycling unit installation (optional)

The Recycling unit is placed in the experimental area, near the gas box unit and as close as possible to the place where the animal will be ventilated, for example in the front side of the Gas Box unit.

Follow the next steps to connect the Recycling unit:

- Plug the pneumatic tube marked as I.05.0010.1.A in the Air_Cont port of the Recycling valve.
- Plug the pneumatic tube marked as I.05.0010.1.B in the Bag_Cont port of the Recycling valve.
- Plug the expiration tube of the main pneumatic valve in the GAS_in port of the Recycling valve.
- Plug the gray tube of the recycling bag (B.03.0000) in the Bag_out port of the Recycling valve.

⚠ NOTE: The Air_out port of the Recycling valve must remain unconnected.



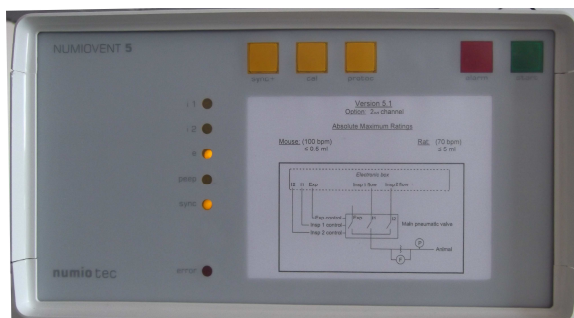
Recycling unit connection

8. OPERATION INSTRUCTIONS

Initial conditions

Before operating the ventilator, follow the next steps:

- Make sure that the pneumatic and electronic connections are according to the directions described in the Installations part of this manual.
- Open the external air pressurized source.
 - ⚠ **WARNING:** use the external pressure values as indicated in the pneumatic specifications table.
- If the automatic refilling unit is used, open the nitrogen pressurized source.
 - ⚠ **WARNING:** use the nitrogen pressure values as indicated in the pneumatic specifications table.
- If the automatic refilling unit is used, open the vacuum source.
 - ⚠ **WARNING:** use the vacuum pressure values as indicated in the pneumatic specifications table.
- Plug the usb cable in the usb port of the external PC.
- Plug the 220V cable in the AC outlet.
- Switch on the ventilator using the switch located in the rear panel of the Main box unit.



Picture of the front panel of the device after executing the initial steps*

* The front panel scheme is printed according to the user configuration.

⚠ WARNING: If the ventilator leds lighting do not correspond to the previous picture, please contact the device manufacturer before proceeding to operate the ventilator.

Software

The Animal Control software (only available in English) starts with a test of the USB ports of the personal computer, to detect automatically the presence of the instrument.

⚠ NOTE: If no device is found or all devices are turned off the software will start in simulation mode.

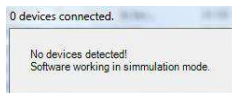
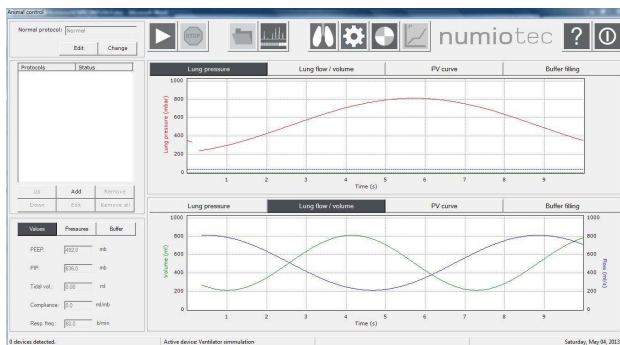


Image of the window open when no devices are detected



Main window of the simulation mode

If the device is connected and turned on, the software will start in the general mode.

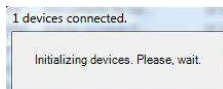
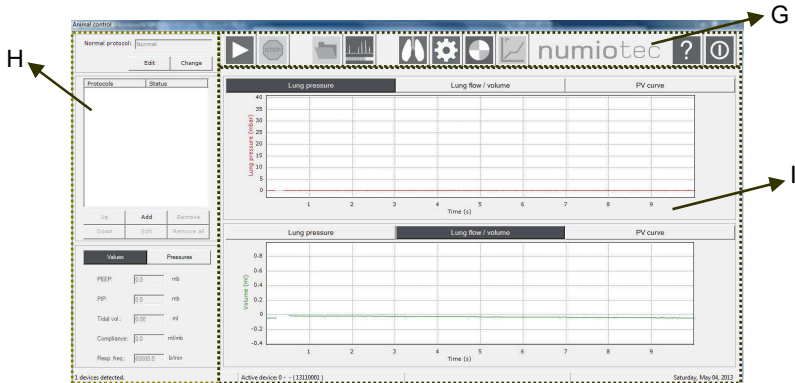



Image of the window open when the device is detected

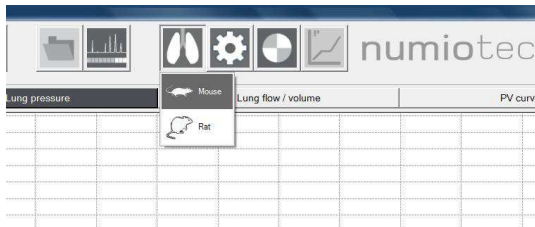
The software is composed of a tool bar with quick access buttons (**G**) and two vertical areas. The left vertical area (**H**) is used to operate the system as well as

to show numerically the acquired and calculated data and the right one (I) is used to visualize graphically the acquired data.



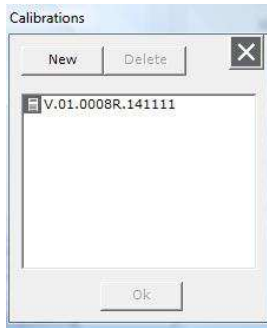
Main window of the general mode

NOTE: Before running the experiments, the user must select the corresponding animal type. Check the *set animal type* button  in the quick access buttons bar.



Selection of the animal type

If the animal type is changed, the software will ask for a calibration file. The name of the calibration file corresponds to the main pneumatic valve to be used and the date of the calibration.




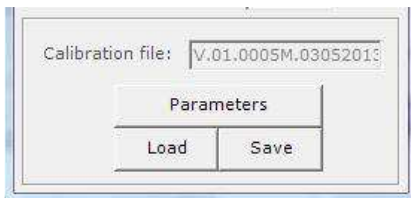
Calibration file loading window

9. CALIBRATIONS

⚠NOTE: The ventilator must be calibrated during installation, once a month (Lung pressure, air inspiration flow and gas inspiration flow procedures) and whenever the measured parameters are not in accordance with the expected ones.

Each main pneumatic valve has a calibration file. This file stores all calibrations, even if they are valve independent. To Load or Save the calibration file:

- Click the *Tools* button  of the quick access buttons tools bar
- Click the *Load* or *Save* button of the *Tools* window.





Loading or saving the calibration file in the Tools window

⚠NOTE: The calibration parameters are accessed by the *Parameters* button. This section is password protected to avoid change by a non expert user and should only be accessed by expert technical personnel.


The calibration procedures can be executed together or independently.


To run the calibration procedures together:


- Click *Calibration* button  of the quick access buttons tools bar.
- Check the desired calibration procedures in the calibration list menu.
- Click *calibrate* button  in the calibration list menu.

A new window indicating the steps to follow will appear.

To run the calibration procedures in an independent way:

- Click *Calibration* button  of the quick access buttons tools bar.
- Check the desired calibration procedure in the calibration list menu.

- Click *calibrate*  in the calibration list menu.

⚠ NOTE: Clicking the *stop*  button stops all automatic procedures. Calibrations are the most common automatic procedures stopped with this button.

⚠ NOTE: After stopping the automatic procedures, the system returns to manual operation.

Next, each calibration procedure will be explained.

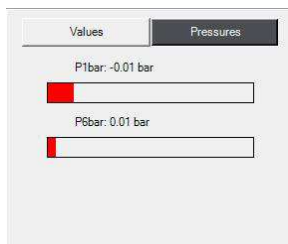
⚠ WARNING: Follow the order described in each case.

Driving gas pressure calibration


⚠ NOTE: The external air pressurized pneumatic tube must be connected to the ventilator and to the external air pressurized source, as indicated in the pneumatic installation section of this manual.

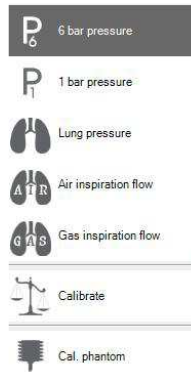
⚠ WARNING: The external air pressurized source must be closed.

- Click the pressures button of the left vertical window. Two red bars indicating the 1 bar (P1bar) and 6 bar (P6bar) pressures will be displayed.




Pressures window with the external pressure closed

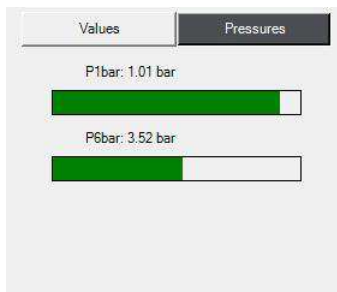
- Click *Calibration* button  of the quick access buttons tools bar.
- Check *6 bar pressure* in the calibration list menu.



Selecting the 6 bar pressure calibration procedure

- Click *calibrate*  in the calibration list menu.
- An indicative window will appear. Please, open the external pressure supply point with the indicated pressure value and press OK.
- An indicative window will appear. Please, open the external pressure supply point with the indicated pressure value and press OK.
- Save the configuration file.

⚠ NOTE: The P6bar bar in the Pressures window is in green color now.




Pressures window with the external pressure open and calibrated

⚠ WARNING: Please contact the device manufacturer before proceeding to operate the ventilator if after the 6 bar pressure calibration and with the external air pressurized source open, the P6bar bar is in red color.

⚠NOTE: The 6 bar pressure is calibrated during installation. The user should only calibrate it when instructed by the manufacturer.

1 bar pressure calibration

⚠NOTE: The 1 bar pressure is calibrated during installation. The user should only calibrate it when instructed by the manufacturer. If calibration is needed, please proceed as in the 6 bar pressure calibration (check *1 bar pressure*  instead).

⚠WARNING: Please contact the device manufacturer before proceeding to operate the ventilator if after the 1 bar pressure calibration and with the external air pressurized source open, the P1bar bar is in red color.

Lung pressure calibration

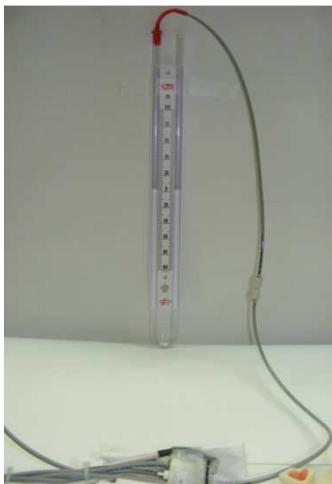
⚠NOTE: The main pneumatic valve must be connected to the ventilator as indicated in main pneumatic valve installation section of this manual.

⚠NOTE: The external air pressurized pneumatic tube must be connected to the ventilator and to the external air pressurized source, as indicated in the pneumatic installation section of this manual.

⚠WARNING: The external air pressurized source must be open.

- Plug the output of the main pneumatic valve to the water column supplied in the Testing and calibration pack.

⚠NOTE: Place the water column vertically and be sure that the water in the column is in the zero line.




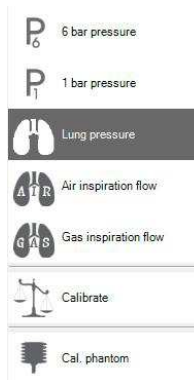
Water column connection in the main pneumatic valve

- Plug a syringe in the pneumatic expiration tube of the main pneumatic valve.




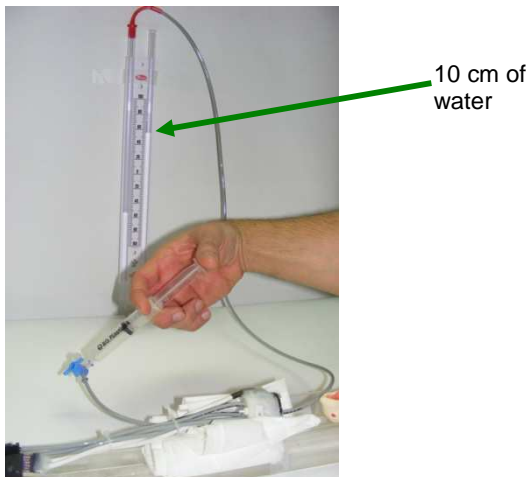
Syringe connection in the expiration tube of the main pneumatic valve

- Click *Calibration* button  of the quick access buttons tools bar.
- Check *Lung pressure* in the calibration list menu



Selecting the Lung pressure calibration procedure

- Click *calibrate*  in the calibration list menu.
- An indicative window will appear. Do not press the syringe and press *OK* button.
- An indicative window will appear. Please, press the syringe until the level difference is 10 cm of water in the column and press *OK* button.



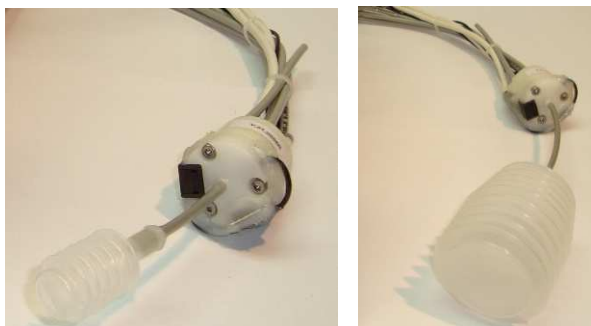
Applying 10 cm of water in the lung pressure calibration procedure

- Save the configuration file.
⚠NOTE: The pressure line in the pressure graph window must be in 10 mbar.


- ⚠ **WARNING:** Please contact the device manufacturer before proceeding to operate the ventilator if the pressure line in the pressure graph window is not in 10 mbar.
- Remove the syringe from the expiration tube of the main pneumatic valve.
- ⚠ **NOTE:** The pressure line in the pressure graph window must be in 0 mbar.
- Unplug the water column from the output of the main pneumatic valve.

Primary gas flow calibration

- ⚠ **NOTE:** The main pneumatic valve must be connected to the ventilator as indicated in main pneumatic valve installation section of this manual.
- ⚠ **NOTE:** The external air pressurized pneumatic tube must be connected to the ventilator and to the external air pressurized source, as indicated in the pneumatic installation section of this manual.
- ⚠ **WARNING:** The external air pressurized source must be open.
- Connect the output of the main pneumatic valve to the phantom supplied in the Testing and calibration pack.
- ⚠ **WARNING:** Select the phantom corresponding to the main pneumatic valve installed.





Plugging the phantom corresponding to the main pneumatic valve (left: mouse, right: rat)


- Click *Calibration* button  of the quick access buttons tools bar.
- Check *Inspiration flow 1* in the calibration list menu




Selecting the Inspiration flow1 calibration procedure


- Click *calibrate*  in the calibration list menu.
- An indicative window will appear. Press *OK* button to start the automatic calibration procedure.
- Save the configuration file.

 **WARNING:** The normal mode will be calibrated according the *normal mode in Channel 2 selection (Tools menu)*. If the user checks that box, the normal mode will be calibrated using the secondary gas.

 **WARNING:** Please contact the device manufacturer before proceeding to operate the ventilator if the difference between the desired and measured lung volumes is higher than the Inspiratory volume accuracy reported in the pneumatic specifications of this manual.

Secondary gas flow calibration


 **NOTE:** The main pneumatic valve must be connected to the ventilator as indicated in main pneumatic valve installation section of this manual.

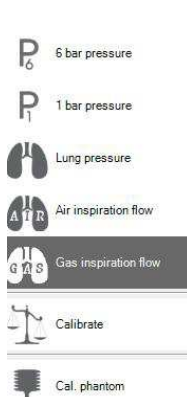
 **NOTE:** The gas box (optional) must be connected to the ventilator as indicated in gas box installation section of this manual.

- ⚠ NOTE:** The external air pressurized pneumatic tube must be connected to the ventilator and to the external air pressurized source, as indicated in the pneumatic installation section of this manual.
- ⚠ WARNING:** The external air pressurized source must be open.
- ⚠ NOTE:** The Nitrogen pneumatic tube (optional) must be connected to the ventilator and to the external Nitrogen source, as indicated in the pneumatic installation section of this manual.
- ⚠ NOTE:** The vacuum pneumatic tube (optional) must be connected to the ventilator and to the external vacuum source, as indicated in the pneumatic installation section of this manual.
- If use and secondary gas channel (no gas box), The secondary gas pneumatic tube must be connected to the ventilator and to the secondary gas pressurized source, as indicated in the pneumatic installation section of this manual


⚠ WARNING: The secondary gas pressure must be in the range specified in the pneumatic specification table (chapter 5)
 - Connect the output of the main pneumatic valve to the phantom supplied in the Testing and calibration pack.


⚠ WARNING: Select the phantom corresponding to the main pneumatic valve installed.
 - Open the nitrogen and vacuum external source (optional).

⚠ WARNING: use the nitrogen and vacuum pressure values as indicated in the pneumatic specifications table.
 - Click *Calibration* button  of the quick access buttons tools bar.
 - Check Gas *inspiration flow* in the calibration list menu





Selecting the Inspiration flow2 calibration procedure


- Click *calibrate*  in the calibration list menu.
- An indicative window will appear. Press **OK** button to start the automatic calibration procedure.
- Save the configuration file.


 **WARNING:** Please contact the device manufacturer before proceeding to operate the ventilator if the difference between the asked and measured gas volume is higher than the secondary gas volume accuracy reported in the pneumatic specifications of this manual.


Buffer volume calibration (Optional)

 **NOTE:** The main pneumatic valve must be connected to the ventilator as indicated in main pneumatic valve installation section of this manual.

 **NOTE:** The gas box must be connected to the ventilator as indicated in gas box installation section of this manual.

 **NOTE:** The external air pressurized pneumatic tube must be connected to the ventilator and to the external air pressurized source, as indicated in the pneumatic installation section of this manual.

 **WARNING:** The external air pressurized source must be open.

 **NOTE:** The Nitrogen pneumatic tube must be connected to the ventilator and to the external Nitrogen source, as indicated in the pneumatic installation section of this manual.


⚠NOTE: The vacuum pneumatic tube must be connected to the ventilator and to the external vacuum source, as indicated in the pneumatic installation section of this manual.


- Connect the output of the main pneumatic valve to the phantom supplied in the Testing and calibration pack.

⚠WARNING: Select the phantom corresponding to the main pneumatic valve installed.

- Open the nitrogen and vacuum external source

⚠WARNING: use the nitrogen and vacuum pressure values as indicated the pneumatic specifications table.

- Click *Calibration* button  of the quick access buttons tools bar.
- Check *Buffer volume* in the calibration list menu

- Click *calibrate*  in the calibration list menu.
- An indicative window will appear. Press *OK* button to start the automatic calibration procedure.
- Save the configuration file.

⚠WARNING: Please contact the device manufacturer before proceeding to operate the ventilator if the difference between the asked and measured buffer volume is higher than the buffer volume accuracy reported in the pneumatic specifications of this manual.

⚠NOTE: The buffer volume is calibrated during installation. The user should only calibrate it when instructed by the manufacturer.

10. BASIC OPERATIONS

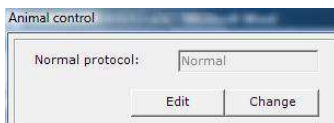
The ventilator is designed to work mainly in two modes: normal and acquisition.

Normal mode operation

The normal mode is an endless breathing protocol in which the animal breathes in two steps: a forced inspiration followed by a free expiration. During the normal protocol, the primary or secondary gas can be supplied to the animal (See Tools menu).

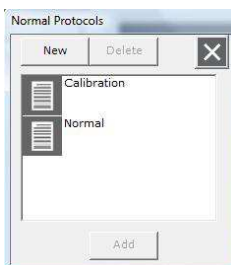
Before operating the ventilator in normal mode, follow the next steps:

- Load the normal protocol parameters clicking the *Edit* button in the normal protocol window of the software:



Normal protocol window

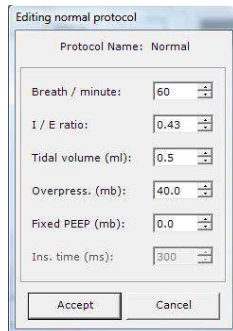
⚠ NOTE: The user can change or delete the loaded normal mode configuration file or create a new one, clicking the *Change* button in the normal protocol window.



Normal protocol change window

⚠ WARNING: Do not delete the Calibration protocol from the list (just used for calibrations purposes).

- Edit the normal mode parameters using the Editing normal protocol window.



Editing normal protocol window

Next, the parameters significance is explained:

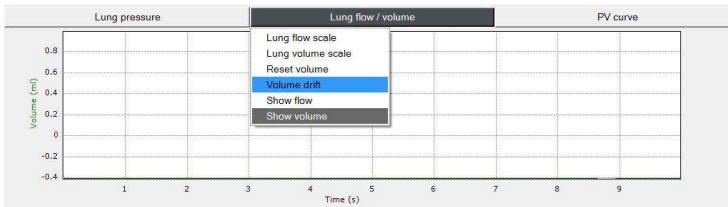
- **Breath / minute:** This is the number of times per minute a breathing cycle is repeated.
- **I / E ratio:** This is the time ratio of the inspiratory / expiratory portion of the breathing cycle.

⚠NOTE: These two parameters (breath per minute and I/E ratio) determine the amount of time the animal is in the inspiratory and expiratory phases according to the relations:

$$T_R = \frac{60000}{\text{breath}} \quad T_E = \frac{T_R}{1 + IE} \quad T_I = IE \cdot T_E$$

Where: T_R is the total breathing time, T_E is the time spent in the expiratory cycle and T_I the time spent in the inspiratory cycle, all times being in milliseconds.


- **Tidal volume:** This is the amount of gas the animal inhales during the forced inspiration.
 - **Overpressure:** This is the maximum allowed pressure. The inspiration valve is closed if lung pressure exceeds this value.
 - **Fixed PEEP:** This is the minimum allowed pressure. The expiration valve is closed if lung pressure lowers this value.
- Adjust the volume drift using the automatic procedure from the *Lung flow / volume* tab in the graphic window of the software.



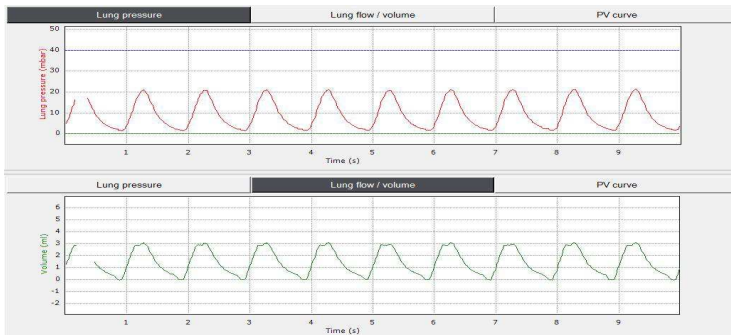
Adjusting the lung volume drift protocol

⚠ WARNING: Please contact the device manufacturer before proceeding to operate the ventilator if the lung volume signal (green line) is not around zero after the volume drift procedure.

The normal mode protocol can be executed clicking the *ventilator on/off* button in the quick access buttons bar

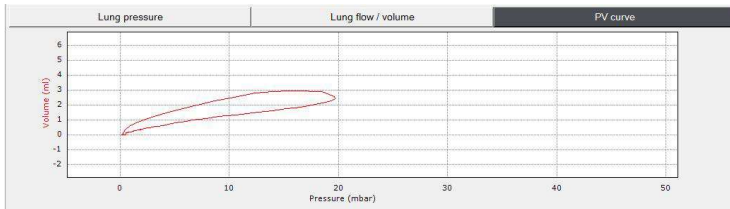
- Click the *ventilator on/off* button to run the normal protocol 

⚠ NOTE: The pressure and volume signals from the animal lung or from the phantom are displayed in the graphic windows of the software.



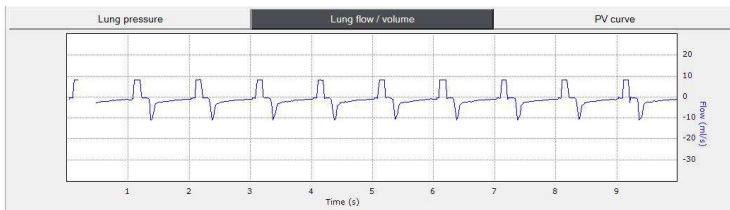
Lung pressure and volume signals in the normal mode protocol

⚠ NOTE: The user can switch between the desired graphics by clicking the corresponding button. The selected plot corresponds to the one in which the button is darker and shows a white font.



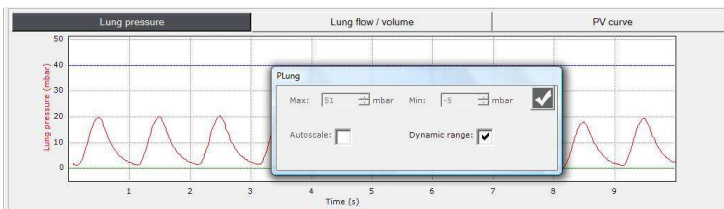
Lung pressure-volume signal

⚠NOTE: In the Lung flow / volume tab, the user can switch between the lung flow signal and the lung volume signal (or both).



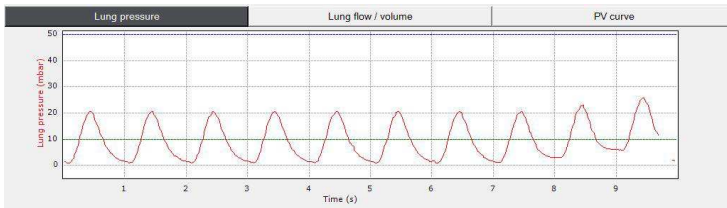
Lung flow signal

⚠NOTE: Clicking the selected plot button displays a menu. Options of this menu include scale change, as well as other options in the lung flow / volume cases. Scaling for each parameter is global (changes in scale are seen in other plots) and maximum and minimum plot values can be set numerically. In addition, a dynamic range in which maximum and minimum correspond to the extreme values which the device can acquire can be selected as well.

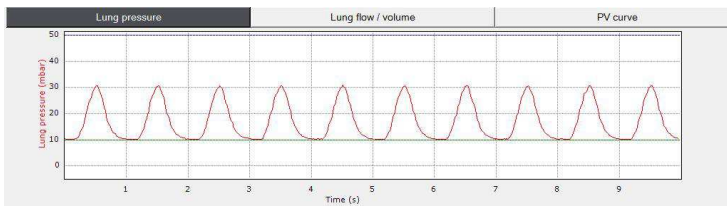


Graphic menu window

⚠ NOTE: When the user selects a Fixed PEEP value in the editing normal protocol window, the software automatically assign the new value of positive lung pressure (green line) and executes an automatic procedure to guarantee that positive value avoiding damage in the lung animal.



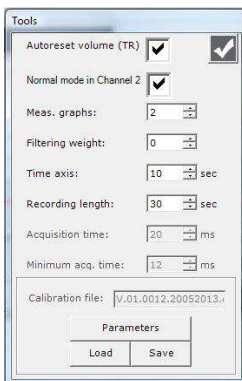
Automatic procedure to assign the positive Lung pressure



Lung pressure after the Fixed PEEP automatic procedure

⚠ WARNING: The highest PEEP value will depend of the PIP signal and the software will force it automatically (green line) after the Fixed PEEP automatic procedure.

⚠ NOTE: Clicking the *tools* button  the user can change functioning and graphic parameters in the tools windows of the software.



Functioning and graphic parameters in the Tools window

Next, the parameters meaning are explained:

- The *Autoreset volume (TR)* choice selects whether the lung volume measurement is reset to zero after every breathing cycle is finished.
- The *normal mode in Channel 2* enables the use of the normal mode with the secondary gas.

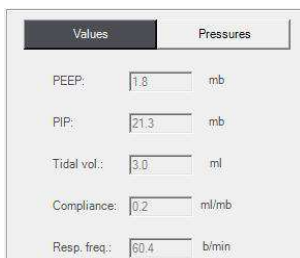
⚠ NOTE: If the user requires the use in the normal mode of the gas supplied in the secondary channel, must check the *normal mode in channel 2* box. In the opposite way, this box must remain unchecked.

⚠ WARNING: When *normal mode in channel 2* box is checked, the software will ask for a corresponding calibration file. Please, select the file according the required gas usage in normal mode. Selecting a wrong file will cause a malfunctioning of the system.

- The *Meas. graphs* box gives the number of graphs that are shown in the main window. Minimum number is 1 and maximum number is 3.
- The *Filtering weight* box selects the filter which is used to graphically show the data. This filter is not used for data which are saved or used for calculations. A Savitzky-Golay filter of the given degree is used.
- The *Time axis* box selects the time length in seconds of data which are shown in the graphs in the main window.

- The *Recording length* box selects the time length in seconds of data which are saved to disk by using the record button.

⚠NOTE: The numerical acquisitions parameters are displayed in the left vertical area of the software general window.



	Values	Pressures
PEEP:	1.8	mb
PIP:	21.3	mb
Tidal vol.:	3.0	ml
Compliance:	0.2	ml/mb
Resp. freq.:	60.4	b/min

Acquired psychological values in the general window

- Click the *ventilator on/off* button  to pause the execution of the normal protocol.

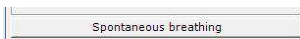
Spontaneous breathing (Optional)

In addition to the normal breathing mode, it is also possible for the animal to breathe spontaneously using its own muscles.

⚠WARNING: In spontaneous breathing mode the animal cannot be paralyzed and anesthesia must be compatible with spontaneous breathing.


⚠NOTE: The animal will breathe spontaneously using the expiration valve whenever the ventilator is not in normal or acquisition mode. In addition to this, it is possible to connect the animal to a gas reservoir (a bag filled with 3He, or with other gas), and let the animal breathe to and from the gas reservoir.


To start / stop this special spontaneous breathing mode, simply press the *Spontaneous breathing* button located below the acquisition protocol panel.



Spontaneous breathing button in the general window

⚠WARNING: The lung pressure control is disabled during spontaneous breathing. As a result, the animal could be damaged if subject to excessive pressure.


 **WARNING:** When the animal is breathing to / from a gas reservoir, carbon dioxide will accumulate in this reservoir. Also, oxygen will be depleted from this reservoir. As a result, spontaneous breathing should not last very long and the animal needs to be monitored while in this state.


 **NOTE:** External trigger and recycling are not compatible with spontaneous breathing.


Acquisition mode operation


The acquisition mode is used for predefined measurements. Acquisition protocols are repeated a given number of times, and during them an animal can breathe from primary gas, secondary gas or both.

An acquisition protocol consists of inspiration, followed by an apnea (period during no gas is allowed in or out the animal), and followed by expiration.

 **NOTE:** Optionally, a trigger signal can be sent to a measurement device during the cycle.

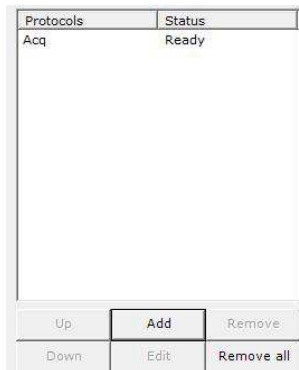
 **WARNING:** Connect the synchronization cable between the ventilator and the image acquisition system as indicated in the electrical installation section of this manual.

 **WARNING:** Consult the image acquisition user's guide to know the synchronism signal characteristics that the equipment needs.

 **WARNING:** See the autonomous mode operation section of this manual for ventilator synchronization pulse type selection.

Before operating the ventilator in acquisition mode, follow the next steps:

- Click the *Add* button in the acquisition window of the software to load new acquisition protocols in the list.

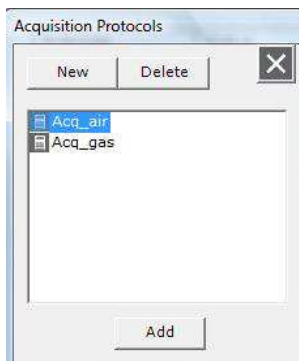


Acquisition window

⚠ WARNING: During the PEEP adjusting automatic procedure, the add button will be disabled.

⚠ NOTE: The acquisition protocols can be loaded and executed in a sequential list shown in the acquisition window of the software. The user can add, edit or remove as many protocols as need.

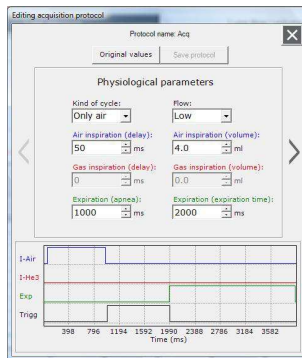
- In the Acquisition Protocols window, click above the desired protocol and press the *Add* button to add to the list.



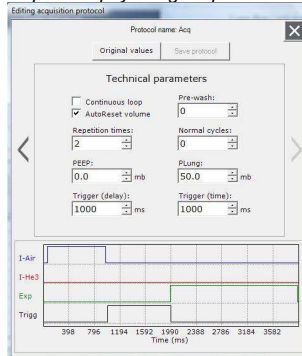
Acquisition Protocols window

⚠ NOTE: The user can delete an existing protocol or create a new one using the *Delete* and *New* buttons respectively.

- In the acquisition window, click on the desired protocol and press the *Edit* button to edit the acquisition protocol parameters. The user can change the physiological and technical values according to the experiment requirements.



Editing acquisition physiological parameters window



Editing acquisition technical parameters window

Next, the parameters meaning are explained:

Acquisition physiological parameters:

- Flow: This can be Low, Medium or High. The amount of flow corresponding to each of these modes depends on the system calibration.
- Kind of cycle: There are four different kinds of acquisition cycles which the device supports. These kinds of cycles refer to whether air or secondary gas is given to the animal, and in which order. In all cases, inspiration precedes apnea which precedes expiration.

The kind of cycles are **Only air**, in which the animal inspires from channel 1 (air), **Air & gas**, in which the animal inspires first from channel 1 (air) and then from channel 2 (secondary gas), **Only gas**, in which the animal inspires from channel 2 (secondary gas), and **Gas & air**, in which the animal inspires first from channel 2 (secondary gas) and then from channel 1 (air).


- Air inspiration (delay): This refers to the amount of time that elapses between the beginning of the cycle and the beginning of air inspiration. During this period of time, all valves (inspiration and expiration) are closed.
- Air inspiration (volume): This refers to the amount of air volume that the animal is given in forced inspiration. During inspiration, the inspiration valve for channel 1 is open, all other valves closed, except if pressure exceeds PLung, in which case all valves are closed. Inspiration flow is constant and depends on the value selected at Flow choice (Low, Medium or High). Volume is adjusted by changing inspiration time. This has no effect if kind of cycle is Only gas.
- Gas inspiration (delay): This refers to the amount of time that elapses between the beginning of the cycle or the end of air inspiration and the beginning of gas inspiration. This has no effect if the kind of cycle is Only Air. During this period of time, all valves (inspiration and expiration) are closed.
- Gas inspiration (volume): This refers to the amount of secondary gas volume that the animal is given in forced inspiration. During inspiration, the inspiration valve for channel 2 is open, all other valves closed, except if pressure exceeds PLung, in which case all valves are closed. Inspiration flow is constant and depends on the value selected at Flow choice (Low, Medium or High). Volume is adjusted by changing inspiration time. This has no effect if kind of cycle is Only Air.
- Expiration (apnea): This refers to the amount of time apnea lasts. During apnea, all valves (inspiration and expiration) are closed. Apnea begins at the end of inspiration.
- Expiration (expiration time): This refers to the amount of time expiration lasts. During expiration, all inspiration valves are closed, and expiration valve is open, except if pressure lowers PEEP, in which case all valves are closed. Expiration begins at the end of apnea.



Acquisition technical parameters:


- Continuous loop: This refers to whether the number of acquisitions will be limited or not. If this is set, acquisition will be performed indefinitely until it is stopped by the user.

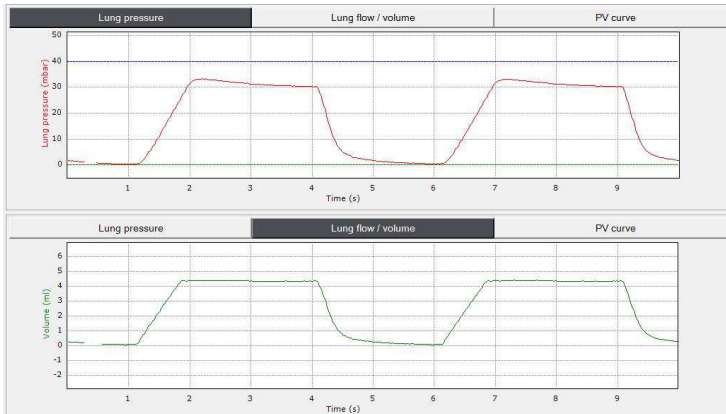
- AutoReset volume (TR): This refers to whether lung volume is reset after each TR. This overrides the selection made in the *Tools* window.
- Repetition times: This is the number of times a given acquisition cycle is repeated.
- Pre-wash: This is similar to the number of repetition times, but in this case the trigger signal is not sent to the measuring device. Prewashes always precede repetitions.
- Normal cycles: This is the number of normal cycles that are executed before the acquisition cycles begin.
- PEEP: This is the minimum lung pressure allowed. The expiration valve is closed if lung pressure lowers this value. The software will assign to this value the same Fixed PEEP value of the previous normal mode.
- PLung: This is the maximum lung pressure allowed. The inspiration valve is closed if lung pressure exceeds this value.
- Trigger (delay): This refers to the amount of time that elapses between the beginning of the cycle and the beginning of the trigger signal.
- Trigger (time): This refers to the amount of time that elapses between the beginning and the end of the trigger signal.

△ **NOTE:** The graphical sequence of the physiological and technical parameters selected is displayed in the lower part of the editing acquisition protocol window.

- Press the *run the list of protocols* button  in the quick access buttons bar to execute the loaded protocols.


 **WARNING:** During the PEEP adjusting automatic procedure, the *run the list of protocols* button  will be disabled, and a message will advise to the user.

 **NOTE:** To execute the acquisition protocols loaded in the acquisition list, the ventilator must be running in a normal mode.




Lung pressure and volume signals in the acquisition protocol

⚠NOTE: The acquisition protocols will execute in the same order as they appear in the acquisition window.

⚠NOTE: If you need, press the *stop the list of protocols* button  in the quick access buttons bar to stop the execution of the acquisition protocols before the acquisition list execution is finished.

Save and process the physiological data

⚠NOTE: Data need to be recorded at inspiration in acquisition mode.

- Click the *record*  button to record physiological data to disk.

⚠NOTE: After recorded, data are stored in a folder named <PROGRAMDIR>\devices\<DEVICEID>\data\<DATASET>

Next, the folder root is explained:

- PROGRAMDIR is the software installation folder.
- DEVICEID the serial number of device which is being used.
- DATASET the name the user gives to a particular dataset.

⚠NOTE: Files within this folder are named data.edf and data.xls and format is European Data Format and Microsoft Excel® Sheet respectively.

Hyperpolarized gas usage (Optional)

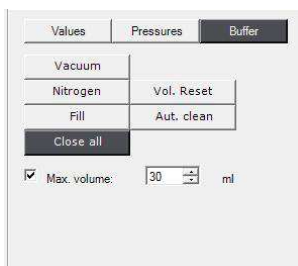
Hyperpolarized gas (e.g. ^3He or ^{129}Xe) can be applied to the animal to perform special MRI experiments. In order to avoid gas depolarization, the gas is sent to

the gas box unit placed inside the MRI scanner (see gas box unit installation), where it is stored until it is applied to the animal.

⚠ WARNING: Before using the software buttons related to the Hyperpolarized gas, be sure that the nitrogen pressurized source and vacuum source are open and the pneumatic connections are in accordance to the instructions explained in the pneumatic installation section of this manual.

To use the Hyperpolarized gas, follow the next steps:

- Press the Buffer button in the left vertical area of the software.



Buffer buttons in the ventilator general window

- Click the buttons according to your needs.
Next, the Buffer buttons functions are explained:

⚠ NOTE: Current open/close status is shown in the buttons themselves, so they appear with a darker color and white font when their mode of operation is selected.

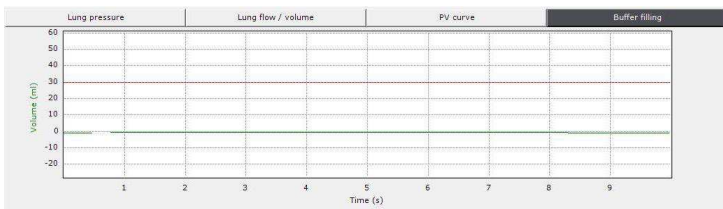
- Nitrogen button is used to fill the tedlar bag with nitrogen.
- Vacuum button vacuums the tedlar bag.

⚠ DANGER: When the system is vacuumed, the helium connector is open. The system must be physically isolated from the ^3He gas cell; otherwise it is possible to accidentally vacuum the Hyperpolarized cell contents. The same is true when cleaning with nitrogen.

- *Fill* button fills the tedlar bag with the Hyperpolarized gas.
- *Close all* button closes the tedlar bag so the system is ready for operation after filling.
- *Aut. clean* button is intended to clean the tedlar bag automatically by repeating several times a cycle in which the tedlar bag is filled with nitrogen and vacuumed.
- *Vol. Reset* button resets the buffer volume measurement to zero.

- **Max. volume** control allows the user to select a maximum volume to be introduced to the tedlar bag. If measured volume exceeds this limit, the tedlar bag is automatically closed.

⚠NOTE: The volume entering the gas bag is measured and displayed by the ventilator in the *Buffer filling* window.



Buffer volume graphic in the ventilator general window

⚠NOTE: Optionally, it is possible to recycle the hyperpolarized gas in a recycling bag. Then the system will send the expired gas to the bag during one minute after the hyperpolarized gas is given to the animal. The recycling bag can be detached and sealed after or during experiment and the gas recovered afterwards.

⚠WARNING: To recycle the hyperpolarized gas, the recycling unit must be installed in the ventilator as indicated in the Recycling unit installation.

Other gas usage if Gas box is installed (Optional)

Other gases (e.g. SF₆/O₂ or air enriched with oxygen) can be applied to the animal to perform special MRI experiments.

⚠WARNING: Before executing the next steps, close the external air pressurized source.

To use another gases, follow the next steps:

- Unplug the black pneumatic tube from the red connector of the gas box.
- Unplug the black pneumatic tube from the gas input of the main pneumatic valve.
- Plug the main pneumatic tube labeled as I.07.00001 between the red connector of the gas box and the gas input of the main pneumatic valve.



Pneumatic connection used in the other gas usage

- Unplug the bridge in the rear part of the ventilator box.
- Connect a 4mm terminal in the air out (1bar) pneumatic port.
- Plug the external source of gas to the gas in (1 bar) port.



Rear panel connections in the other gas usage

- Open the external air pressurized source and the other gas source.
 - Load the calibration file corresponding to this kind of experiments.
- ⚠ WARNING:** The calibration file must be different than the one used with the gas box. If necessary, calibrate the inspiration flow 2 as indicated in the calibration section of this user's guide.

Autonomous mode

To allow the use of the ventilator in the autonomous mode (without PC), the front panel is provided with 6 leds and 5 buttons. The buttons are used to execute the main process stored in the internal memory of the ventilator.

⚠ WARNING: Before proceeding to use the front buttons, load the desired normal parameters and acquisition protocols in the ventilator software.

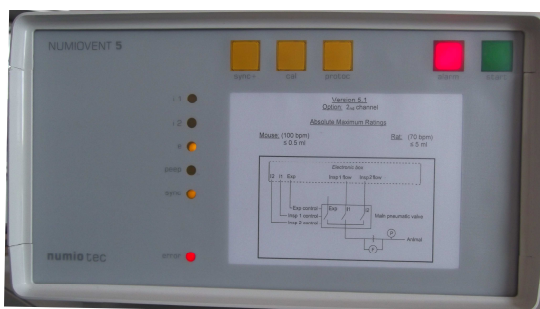
Next, the ventilator front buttons functions are explained:

- The *start* button is used to execute the normal mode sequence with the parameters stored in the internal memory of the ventilator. During the execution the green led is illuminated.



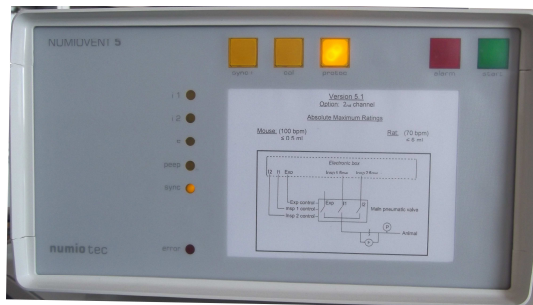
Front buttons of the ventilator and the start button activated

- The *alarm* button is used to silence the beeper sound activated when the lung pressure of the animal is higher than the overpressure selected by the user. In that state the red led of the button is illuminated.



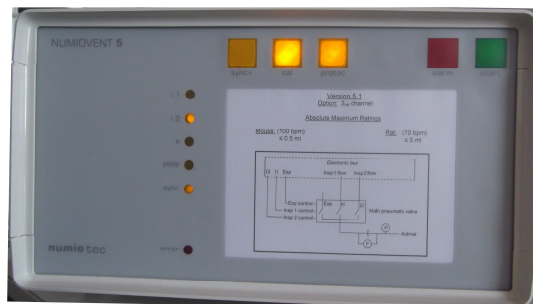
Alarm button activated

- The *protoc* button is used to execute the list of acquisition protocols stored in the internal memory of the ventilator. During the execution of the protocols the yellow led of the button is illuminated.



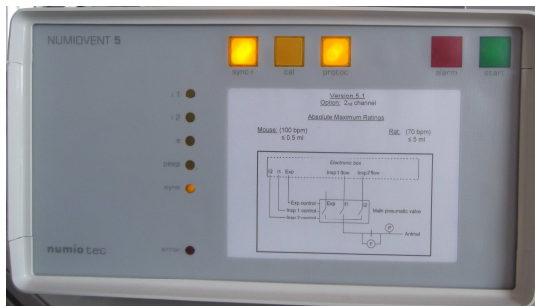
Protoc button activated

- The *cal* button is illuminated when the ventilator is being calibrated.



Cal button activated

- The *sync+* button is used to switch between the positive and negative synchronism signals used to control, for example, imaging devices according to the breathing pattern applied to the animal (See the acquisition mode section of this manual). The normal condition is a negative synchronism signal and the yellow button led is not illuminated. When selecting positive synchronism the yellow button led is illuminated.

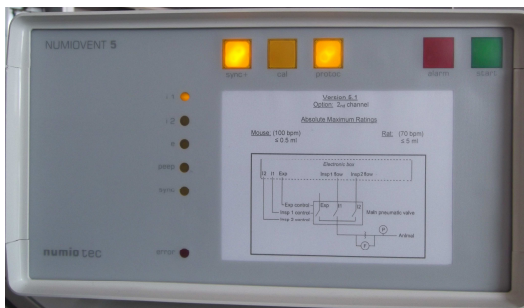


Sync+ button activated

Six leds are used to visualize the real time state of the process that are being executing in the ventilator.

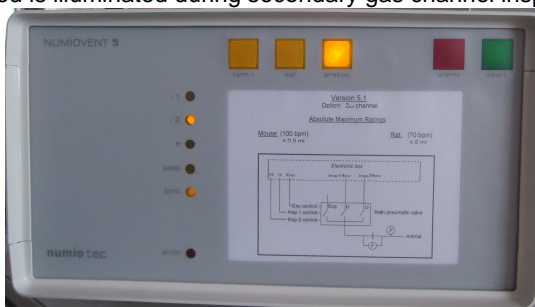
Next, the ventilator frontal leds functions are explained:

- The **i1** led is illuminated during air channel inspiration time.



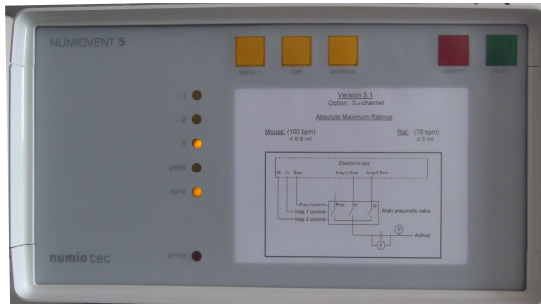
Channel 1 inspiration time

- The **i2** led is illuminated during secondary gas channel inspiration time.



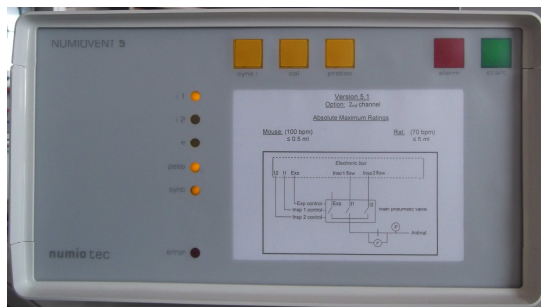
Channel 2 inspiration time

- The **e** led is illuminated during animal expiration time and when no sequence is running (spontaneous breathing mode).



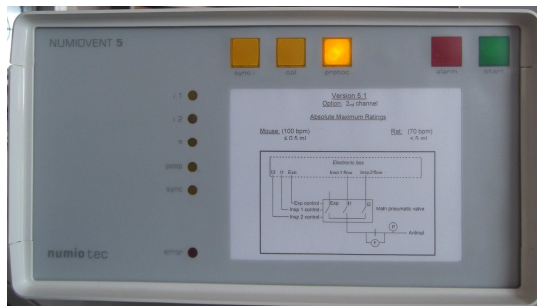
Expiration time

- The **peep** led is illuminated during the time that the lung pressure is lower than the established fixed peep pressure selected in the software.



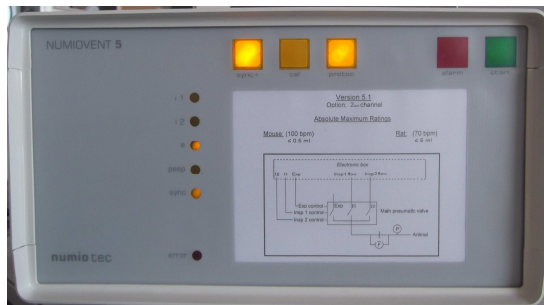
Peep time

- The **sync** led changes state during the synchronism pulse sent by the ventilator to the Image Acquisition machine. If the user selects negative synchronism pulses (**sync+** button OFF), the **sync** led goes out during the pulse.



Negative synchronism time

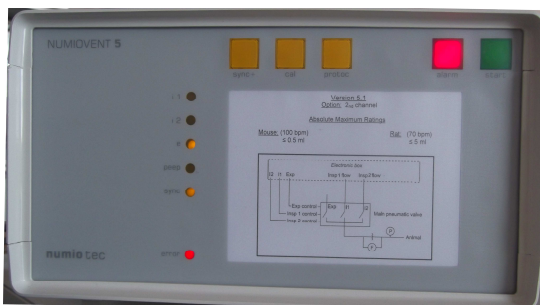
⚠ NOTE: If positive synchronism pulse is selected (sync + button ON), the sync led is illuminated during the pulse duration.



Positive synchronism time

⚠ WARNING: Before proceed to select the synchronism pulse type, consult the image acquisition user's guide to know the synchronism signal characteristics that the equipment uses.


- The **error** led is illuminated during the time that the lung pressure is higher than the established overpressure selected in the software.




Overpressure time


11. AFTER USE INSTRUCTIONS


Once finished to work with the ventilator, please follow the next instructions:


- Close the software in the exit  button.
- Switch off the power supply using the switch in the 110-220V connector.
- Close the nitrogen and vacuum pressurized source (if used).
- Close the external air pressurized source.

 **DANGER:** Never close the driving gas source before the other pressurized sources used in the system. It could damage seriously some pneumatic parts of the ventilator.

12. CLEANING

 **WARNING:** Electric shock hazard. Do not remove outer case of this unit. All dismantling and maintenance of this unit must be done by a qualified service technician. Refer servicing to qualified service personnel.


 **WARNING:** This unit does not require oil. Do not attempt to lubricate any internal parts.

 **WARNING:** Unplug unit before cleaning. Do not submerge in water for cleaning.


Please follow the next instructions to clean the ventilator:

- Wipe the main unit with a damp cloth every few days to keep it dust-free.
- Do not use any powdered detergents or soap. Do not submerge the unit into water.

13. MAINTENANCE

 **CAUTION:** To reduce the risk of electric shock, do not disassemble the unit.

All repair services and maintenance procedures must be performed by qualified service personnel.

 **NOTE:** If the user opens the main box unit without the authorization of the manufacturer, the warranty will void.

14. TROUBLE SHOOTING

If your ventilator fails to function, consult the trouble shooting guide below. If the problem persists, consult your equipment provider.

Device Doesn't Work.

- Check if the power cord plug is properly fitted into an appropriate electrical outlet.
- Check if the power switch is turned to ON position.
- Check for correct electrical voltage.

Device is switched ON but the software doesn't Work.

- Check if the usb cable is properly connected to the usb port of the ventilator and the PC.

Device is switched ON but the signals don't appear correctly in the graphic section of the software.

- Check if the Main pneumatic valve & pressurized buffer and Tedlar bag module sensors cable is connected between the ventilator and the main pneumatic valve and the gas box.

For other fails, please contact with the technical service of NumioTecnologías S.L.