Thank you for your purchase of Anesthesia equipment for test animals at this time.

Prior to using these products, read this instruction manual for sure to utilize full-fledged performance of these products and ensure customer’s safety.

Retain this manual in an easily accessible place near a system for future reference.
1. List of Parts

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Reference
### Caution

**Note:** Device for investigational use in laboratory animals or other tests that do not involve human subject.

**Application:** This anesthetic system for test animal is designed for IV100/OV100. If this system is used with other method than those described in this manual, be advised that the safety cannot be guaranteed and; it may cause a failure. Use this system in accordance with this manual.

**Note:** Anesthesia may result in a different reaction if the animal under test is different even if it is tested in the same anesthetic condition. When using this system, it is essentially required that the oxygen flow rate and concentration of anesthetic gas to be supplied are properly adjusted by monitoring physiological response of the animal under test. This product includes natural rubber latex which may cause allergic reaction. When handling Small Rodent Face Mask, use a protector like gloves and do not touch the product directly with your hand.
1. **List of Parts**
   See Fig. 1, 2, 3 and 4.

1:  3 meter long X 1/4 inch ID Green nylon reinforced color coded high pressure oxygen hose
1a: Female DISS O2 Hose Assembly
1b: Female DISS O2 Hose Assembly
1c: Male DISS O2 threaded X 1/8 NPT Male threaded

2: "PAM" Portable Anesthesia Machine
2a: 0 - 1 lpm O2 Flowmeter
2b: Mechanical Stop O2 Flow Control
2c: 1/8 NPT Male threaded X 90 degree 1/4 inch Hose Barb
2d: 3/16 inch ID X 30.5 cm Silicone Tubing--connects outlet of flowmeter to vaporizer inlet adapter (2E)
2e: Vaporizer Inlet Adapter
2f: Vaporizer Outlet Adapter
2g: 3/16 inch ID X 30.5 cm Silicone Tubing--connects outlet of vaporizer to Dual Diverter Valve (4)
2h: Common Outlet (Fresh Gas Outlet) of PAM color coded with a white band around its body.
2i: PAM Handle
2j: 1/4 X 20 Winged Thumb Screw for body of Dual Diverter Valve
2k: 1/4 X 20 Winged Thumb Screw for Stainless Steel Rod (4h)

3: Tec 3 Style Isoflurane Pin Indexed Precision Vaporizer (see Operator Manual for Vaporizer)
3a: Isoflurane Pin Index Fill Device (see instructions inside Isoflurane Pin Index box)

4: Dual Diverter Manifold with 2 color coded Stopcocks and clear tubing with color coded Common Outlets attached.
4a: Stopcock which controls flow of gas to Induction Chamber (5) color coded with a purple band around its body.
4b: 2 meter X 6mm ID Clear Vinyl Tubing for the inlet of the Induction Chamber.
4c: 6mm male X 15mm male adapter color coded with purple band for fresh gas inlet of induction chamber (5a).
4d: Stopcock which controls flow of gas to Non-Rebreathing System (6 or 7) color coded with a green band around its body.
4e: 2 meter X 6mm ID Clear Vinyl Tubing for the inlet of the Non-Rebreathing System.
4f: Common Outlet (Fresh Gas Outlet) for Non-Rebreathing System color coded with a green band around its body.
4g: 6mm male X 15mm male adapter color coded with a white band for Common Outlet of PAM (2h).
4h: 3/8 inch X 8 inch Stainless Steel Mounting Pin for Dual Diverter Manifold
5: PosiSeal Mouse Induction Chamber
5a: 15mm female Inlet Adapter for fresh gas into the Induction Chamber color coded with a purple band around its body.
5b: 15mm female Outlet Adapter for waste anesthetic gas out of the Induction Chamber color coded with a blue band around its body.
5c: Induction Chamber Lid Latches

6: Universal Rodent Nosecone Non-Rebreathing System
6a: Universal Rodent Nosecone (Fresh Gas Reservoir)
6b: Universal Rodent Nosecone Body. It is color coded with a yellow band around it.
6c: Fresh Gas Feed Tube for URN NRB System color coded green.
6d: 3mm male X 15mm male adapter color coded with a green band around its body.

7: Mapleson-D Non-Rebreathing System  [Consumable: PN AA-00-0500-07]
7a: Small Rodent Face Mask
7b: Body of Mapleson-D Non-Rebreathing System color coded with a yellow band around its body.
7c: 6mm ID X 20.3cm clear vinyl feed tube for Mapleson-D.
7d: 6mm male X 15mm male adapter color coded with a green band around its body.

8: 2 meter X 6mm ID clear vinyl tubing for the Waste Gas Evacuation from Non-Rebreathing systems.
8a: 6mm male X 19mm female adapter color coded with a yellow band around its body.
8b: 6mm male X 22mm female adapter identified with the verbiage "To F/Air Canister."

9: 2 meter X 6mm ID clear vinyl tubing for the Waste Gas Evacuation from the Induction Chamber.
9a: 6mm male X 15mm male adapter color coded with a blue band around its body.
9b: 6mm male X 22mm female adapter identified with the verbiage "To F/Air Canister."

10: F/Air Canister  [Consumable: PN AA-00-0581-01]

TIP: Regarding consumable purchase, contact Olympus Local Representatives.
Fig. 4
2. Preparation for Anesthesia
2.1. Filling (Draining) of Anesthetic Solution to (from) Anesthetic System

Note: Install the anesthetic system on flat surface and be sure that it is not inclined. Do not move vaporizer by holding the dial control only. Be sure to move the vaporizer by holding PAN Handle (#2i).

To turn vaporizer “ON”, depress the dial control release button and turn the dial in counter-clockwise direction. With this way, the anesthetic solution will be vaporized.

When the vaporizer is not in use, set the dial control to “OFF” in a way that the anesthetic gas does not come out inadvertently.

Note:

・ Do not fill any solution other than Isoflurane specified on front label of the vaporizer. The vaporizer is designed and dedicated for Isoflurane. If the solution other than Isoflurane is used, it would cause a failure of the vaporizer or it would pose hazard to animal under test.
・ When anesthetic solution is filled in vaporizer, turn the dial control to OFF position. When the solution is being filled, anesthetic gas may leak out.
・ During filling, do not turn the dial to ON position. In addition, be sure that the anesthetic solution is not filled any more than maximum level mark (△) and it is not spilled over.
・ Dispose of solution waste in accordance with Waste Liquid Treatment Regulation in each facility.
・ Check level of the solution being used periodically. In order not to degrade performance of the vaporizer, the solution should remain above the minimum level mark (▽) of the solution level indicator.
・ When anesthetic solution is filled in the vaporizer, do not incline the anesthetic system.
Filling anesthetic solution agent requires the following items.

- Anesthetic Solution Agent (Isoflurane Solution Bottle) : Prepare on customer's side.
- Key-fill Device dedicated for Isoflurane
- Filling/Draining Unit (Keyed Filler) attached to vaporizer

### 2.1.1. Filling Anesthetic Solution

1. Remove cap and seal of the anesthetic solution bottle. Check whether or not the bottle has any chipping or crack. Align key slot of Key-fill Device with key slot of the bottle collar and turn it until it is securely tightened.
   
   **TIP:** Key-fill Device does not fit to any anesthetic solution bottle other than Isoflurane.

2. Verify that the dial control is set to OFF position. Turn the fixing screw located on top of the filler unit to counter-clockwise direction and extract the dummy filler plug.

   **Note:** The vaporizer may be pressurized. When the dummy filler plug attached to key slot of the vaporizer should be removed, turn the top fixing screw slowly.

3. Making two holes of Key-fill Device to face downwards, insert the Key-fill Device into filler socket.

4. After insertion, turn the top fixing screw in clockwise direction to tighten and then, snug-fit the Key-fill Device into filler socket.

![Fig. 7 Keyed Filler Components](image)

5. Raise the bottle above the level of the filler socket. At this moment, ensure that Key-fill Device tube is not kinking. Within two seconds, bubbles would appear from Key-fill Device inner tube regularly. If the bubbles do not come out, remove the bottle together with Key-fill Device from vaporizer and detach the Key-fill Device from the bottle. Shake the adapter two or three times carefully to repel anesthetic solution from tube and then, attach the Key-fill Device to anesthetic solution bottle again.

6. When the vaporizer is filled to the maximum-level mark (▲) of the level indicator, lower the bottle below the level of the filler socket and wait for about 5 seconds and, after any of the agent inside Key-fill Device is returned back to the bottle, loosen top fixing screw and remove the Key-fill Device from the filler. If any extra agent is dripping from the filler socket, wipe it out from the filler socket completely. In order to prevent gas from leaking through filler, insert dummy filler plug and tighten it completely.

   **TIP:** In case the vaporizer is empty before filling, the anesthetic level may be decreased slightly because the wick inside the vaporizer may absorb the agent.
2.1.2. Draining Anesthetic Solution
1. Attach the Key-fill Device to empty bottle. Making two holes of the Key-fill Device to face upward, insert it into the drain socket. Tighten the bottom fixing screw.
   TIP: Only Key-fill Device dedicated for Isoflurane can be fitted to the drain socket.
   Note: The vaporizer may be pressurized. Turn the top fixing screw slowly.

2. Ensure that the bottle is positioned below the drain socket and the tube is not kinking. For draining (and for air ventilation), loosen the top fixing screw and remove dummy plug from the filler socket.

3. Turn the drain valve in counter-clockwise direction and open it so that anesthetic solution is drained from the vaporizer.

4. If the draining process cannot be done completely, close the drain valve and unscrew the bottom fixing screw and then, remove the bottle together with the Key-fill Device. Shake the Key-fill Device two or three times carefully to repel anesthetic solution from tube and then, attach the Key-fill Device to anesthetic solution bottle again.

5. When draining is completed, close the drain valve (clockwise direction), unscrew the bottom fixing screw and remove the bottle and adapter. Fit dummy filler plug to the filler socket again and tighten the top fixing screw fully in clockwise direction.

2.2. Changing Diaphragm/Making Anesthetic Mask
2.2.1. Change of O-ring and Diaphragm
1. Silicon O-ring serves for holding a diaphragm in specified place of Universal Rodent Nosecone or Small Rodent Face Mask.
   Note: If any crack or flaw appears in O-ring, it is not possible to hold a diaphragm in place. Change it. One spare O-ring comes with each kit.

2. O-ring can be removed by rolling it from the groove where it is attached. To remove from the groove, press it with your thumb and roll it forward so that it would come out (see Fig. 8-10). Discard the defective diaphragm and attach a new diaphragm.

3. The diaphragm is made of 0.3mm thick latex sheet. Spare diaphragm material comes with each kit.

TIP: O-ring is a part of the Small Rodent Face Mask (articles of consumption).
2.2.2. Making Anesthetic Mask
1. Stretch a new diaphragm material over the end of anesthetic mask cone (see Fig. 11).
   a. Hold the new latex diaphragm material with your thumb and forefinger of one hand (see Fig. 11).

2. Set the O-ring over the new diaphragm material again and retain it in the groove of anesthetic
   mask cone correctly (see Fig. 12).
   a. Make it sure that no gap exists around periphery of the diaphragm as trace anesthetic gas may escape (see Fig. 13).

3. Pull edges of the diaphragm material to flatten out the diaphragm and allow it to have slight tension (see Fig. 14).

4. Using a pair of scissors, cut off excess diaphragm around the O-ring. The diaphragm material
   remained should be kept for subsequent changes of the diaphragm (see Fig. 15-16).

5. Using a pair of scissors, cut a circular hole in the diaphragm with appropriate size that nose or
   nose-and-mouth of a small animal under test can fit (see Fig. 18-20).
   a. Make it sure that the circular hole is positioned in such a way that, when the muzzle of a
      small animal under test is inserted into the diaphragm, the head of a small animal is relatively
      in line with the body. The anesthetic mask cone is capable of rotating on its axis so that the
      circular hole can be positioned in the diaphragm properly.
   b. The circular hole should be made small enough in the diagram so as to serve as a seal
      around the muzzle of a small animal under test.
   c. The circular hole should be made large enough in the diagram in order not to block nares
      and voluntary breathing.

**Note:** If the diaphragm is cut in cross (+) or X character, it would not serve as a proper seal
   around the muzzle of an animal and anesthetic gas may leak out in work place. Cut a
   circular hole in the diaphragm.
3. Anesthetizing

Anesthesia can be induced in a single animal or in several rodents, using an induction chamber.

3.1. Procedure to Use Anesthesia

TIP: Prepare oxygen tank and regulator on customer's side.

A mouse is anesthetized.

1. Check the state of anesthetic system.

   Note: Verify that there is sufficient gas in the tank until processing is completed.
   ・ Check level of the liquid through a glass window of the anesthetic vaporizer and verify that there is sufficient Isoflurane solution in it.

   Check all connections and make it sure that input to induction chamber (2 diverter valve) is in the open position.

   Note: In 2 diverter valve, connections between induction chamber and anesthetic mask are color coded. Check connections and use them correctly.

2. Set the 2 diverter valve in close position to supply anesthetic gas to induction chamber.

3. Set the oxygen regulator so as to have the adjusting pressure to 0.34MPa or less and turn on the oxygen source. Set the flow rate to induction chamber to 1 liter per minute (1LPM) using flow meter control knob.

   Note: When oxygen is running, do not close both Stopcocks. Connecting tube inside anesthetic system may be disconnected.

4. Put a mouse inside the induction chamber and close the lid of chamber firmly.

5. Turn on vaporizer and adjust Isoflurane concentration to 4-5% (set the concentration lower to 3.5% or so in case of sick or old animal). After about 2 minutes, the mouse will lose its righting reflex and lie down. Lower Isoflurane concentration to 2.5% and let the mouse sleep for further one minute in induction chamber.

6. When the mouse is anesthetized and, if it is entered in moderately deep sleep (lying on its side and breathing rhythmically), remove it from induction chamber and close the lid.

7. Close the gas flow to the induction chamber. If another mouse exists inside the induction chamber at this moment, do not close the gas flow.

8. Insert nose of the mouse into anesthetic mask and open the gas flow to mask side.

9. Adjust O₂ flow to 0.5LPM and reduce Isoflurane concentration to 2.5 or 3.0% (or lower further to maintain surgical plane of anesthesia).

   Gas flow should be set to higher volume than one-time respiration of a mouse. It is set for a purpose to ensure that the exhausted CO₂ from the mouse flows towards anesthetic gas filter without fail.

   Minimum flow rate for mouse recommended: 0.5LPM

   Note: If the anesthetic system is used with lower flow rate than the recommended rate, CO₂ may remain inside the anesthetic mask.
10. Monitor depth of the anesthesia throughout the works all the time.

Note: Anesthesia must be monitored completely from time of induction to full recovery. Animal is judged to have recovered from anesthesia when it is fully awake and it can lie down on its belly. The effectiveness of monitoring is determined by physiological parameters to be monitored. Commonly used parameters are; respiration rate and pattern, heart rate, color of the footpads, response to toe-pinch reflex and painful manipulations. Respiration rate changes from fast respiration during induction to slow, deep and rhythmic respiration. Heart rate follows a similar pattern. In addition to respiration rate and heart rate, other parameters, i.e., response to toe-pinch reflex (leg withdrawal) or color of the footpads (pink in most case of rodents) must be monitored.

11. When works are completed, turn the dial control of the vaporizer to OFF position to shut off the delivery of anesthesia and continue to run oxygen until the mouse is awaken.

12. Close the adjusting valve of regulator and shut off the oxygen flow.

13. Return the animal to cage.

Note: Put a mouse that is recovered from anesthesia in the cage where a gauze or paper towel is lined together with other mouse awaken from anesthesia or other mouse that is anesthetized in the same condition to prevent injuries and inhalation of bedding material unconsciously (to prevent aspiration pneumonia).

14. Check the flow meter and ensure that the oxygen flow is stopped. Turn the control knob of flow meter to OFF position (close fully in clockwise direction).

Note: The problem associated with induction chamber is a possibility of room pollution or personal exposure to inhalation of anesthetics. It occurs when the induction chamber is opened for putting an animal in or removing an animal from it. For this reason, the induction chamber must be placed in a place of good ventilation. When anesthetic mask should be used, be sure that it is snug-fitted to muzzle of the mouse firmly to prevent gas leakage and a hole of appropriate size is cut out in a fresh diaphragm material.

3.2. Precaution in Use of Anesthesia
3.2.1. Anesthetic Gas Absorption Filter: Consumable
F/Air Canister should be replaced when the mass is increased by 50g. Before using F/Air Canister, measure the weight beforehand. F/Air Canister of unused state weighs approximately 300 grams. Record the weight and the date on which the weight was measured on a marginal place of F/Air Canister label. Measure the weight periodically. When the weight of F/Air Canister is increased by 50 grams, discard the said F/Air Canister and install a new F/Air Canister.

Important: F/Air Canister must be installed in lateral direction in order to pass mixed gases (oxygen, CO₂ and Isoflurane) throughout F/Air Canister. Isoflurane would be absorbed by active charcoal but oxygen and CO₂ are not absorbed and they would pass through. The CO₂ emerging from a small animal under anesthesia is not dangerous to human health.

3.2.2. Check before Start
The most problem with anesthetic system is leak. The leak occurs in place related with connection of tubing, flow meter valve and O2 yoke. The anesthetic level will get high to user if a leak occurs and it must be prevented.

Be sure that any tube is not unplugged or any crack occurred on tube.
Reference

Principles of the Anesthetic System

The anesthetic system supplies gases to induce an animal in a deep sleep and release it from pain.

The basic anesthetic supply system is comprised of oxygen (O2) delivery unit, O2 flow meter, vaporizer that produces a vapor from a volatile liquid anesthetic agent, breathing circuit for a small animal under test (tube, connector and valve) and air piping device that repels any extra anesthetic gases. This configuration is very important, since room pollution with anesthetic gases may lead to health problems in animals as well as in humans.1

While the anesthetic gas is supplied to a small animal, O2 runs through vaporizer and it meets with vaporized anesthetic agent to be mixed up. The gases of which O2 and anesthetic agent are mixed up enter lungs of a small animal through the breathing circuit, usually by spontaneously arising circulation (respiration). In case by case, it is required to use assist ventilation, especially when opening the chest (thoracic) cavity. As assist ventilation, a ventilator or respirator is used.

OXYGEN SUPPLY As portable or stationary cylinder stores gas at very high pressure (14MPa), the pressure should be reduced to the level, e.g., 0.34Mpa with which a flow meter can afford. For this reason, a pressure-reducing valve or regulator is required. If a further reduction in pressure is required to supply the gas safely to a small animal, it should be adjusted by use of control knob provided on the flow meter.

Since O2 is regularly consumed, the pressure inside the tank declines linearly. The regulator adjusts automatically when the pressure inside cylinder falls as the gas is used.

1 The gas scavenging system is to eliminate undesired waste anesthetic gases (WAG) from working place and minimize the potential that humans breathe the said gases. WAGs are eliminated with F/Air Canister. During induction at induction chamber and maintenance of the anesthetic system, if the anesthetic mask comes loose or undesired gas leaks out from breathing circuit into the room or spillage or gas escape occurs when filling the vaporizer, pollution may occur. To reduce pollution when using the induction chamber, scavenge the chamber adequately. When an animal is anesthetized, remove it quickly form the chamber and close the lid.
OXYGEN FLOW METER  
This device is provided with a feature to indicate flow rate with use of needle valve since it delivers desired amount of oxygen to the circuit of an animal under test in unit of milliliters or liters per minute. Flows of 0.5 to 1 liter per one minute are commonly used volume for mouse anesthesia equipment. Flow meters are individually calibrated for a specific gas, e.g., oxygen or nitrous oxide. Flow is read with the position where a metal ball exists on the calibrated scale located in the middle of the indicator.

ANESTHETIC VAPORIZER  
The vaporizers produce a gas of accurate concentration from a volatile liquid anesthetic. Using dial or knob on the vaporizer, it is possible to know the ratio precisely by adjusting the anesthetic gases that leave vaporizer. Adjusting with dial or knob, it is possible to know an accurate ratio of anesthetic gases being discharged. The latest vaporizers put on sale by Summit Anesthesia Solutions are accurate at oxygen flow rate of 0.3 liter to 15 liters per minute and it can adjust anesthetic concentration automatically for ambient temperature fluctuation. Since these are precise instruments, it is recommended that these are serviced and calibrated every 3-5 years.

BREATHING CIRCUIT FOR SMALL ANIMAL  
Breathing circuit is the highway to deliver anesthetic gas to a small animal. The purposes of anesthetic breathing circuit are;

A. Supply oxygen to a small animal under test
B. Supply anesthetic to a small animal under test
C. Remove carbon dioxide that is produced by a small animal under test
D. If required, provide a method to assist or control respiration.

Non-rebreathing system is normally used for anesthetizing rodents. It uses fresh gas flows of very high pressure and delivers anesthetic gases and also washes out the exhaled CO2. The set up of this system includes an installation of exhaust hose to scavenge excess anesthetic gases.

When the oxygen is turned on, the gas will flow continuously as follows:

O2 Gas tank → Regulator → Flow Meter → Vaporizer → 2 Diverter Valve → Breathing system for a small animal → Scavenging system

POTENTIAL PROBLEMS WITH NON-REBREATHING SYSTEMS  
The O2 flow rate should be around 3 times the small animal’s minute ventilation. \(^2\) In practical terms, O2 flow rate should be no less than 0.5LPM. When a mouse is anesthetized in induction chamber, the O2 flow must be increased to approximately 1 liter per minute. If the flow is insufficient, CO2 will be absorbed once again and it would cause respiratory acidosis. For this reason, the Non-rebreathing system consumes a great amount of oxygen when it is compared with Rebreathing system used for larger species.

Since there is no rebreathing, the non-rebreathing circuits deliver high flow of dry cool gas to small animals, which causes to take heat and humidity from the body. Small rodents will lose the body heat and dehydrated. It is therefore necessary to place rodents on a heated surface or near a heat source when the processing takes a long period of time. When a blanket with heater or a lamp for heater is used, ensure that the animals are not exposed to heat excessively to cause the blood pressure drop or skin burns. In addition, anesthetized animals would deteriorate the ability to adjust body heat. For breathing circuit of small animal, humidifier may be used to add moisture to the gases.

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\(^2\) Minute ventilation is the volume of air that ventilates the lungs in one minute. It is calculated by multiplying the tidal volume (air moved in and out the lungs with each breath) by respiratory rate (200 to 300 breaths/minute in the awaken mouse).