

## Protocol for XIC-3 Isolation Chamber for use with the IVIS Spectrum.

The XIC-3 animal isolation chamber is a sealed box that provides biological isolation of anesthetized mice while they are being imaged. This is accomplished by filtering the gas going into and coming out of the chamber through two Gibeck Isogard HEPA filters, which prevents the interior of the XIC-3 chamber from being contaminated by potential pathogens. The isolation chambers are available for experiments involving immunocompromised animals. The isolation chambers **ARE REQUIRED** for experiments involving biohazards i.e. bacteria or viruses. The XIC-3 chamber can only be used for bioluminescent and epi-fluorescent imaging it cannot be used for fluorescent trans-illumination imaging.

**POWDER-FREE GLOVES SHOULD ALWAYS BE WORN WHEN HANDLING THE XIC-3 ISOLATION CHAMBER TO PREVENT FINGER PRINTS AND SMUDGES ON THE ANTI-REFLECTIVE WINDOW OF THE CHAMBER.**

**WHEN USING THE XIC-3 ANIMAL ISOLATION CHAMBER THE “GAS OUT” PORT ON THE REAR OF THE IVIS SPECTRUM MUST NOT BE CONNECTED TO THE XGI-8 ANESTHESIA SYSTEM.**

The following procedure will detail how to:

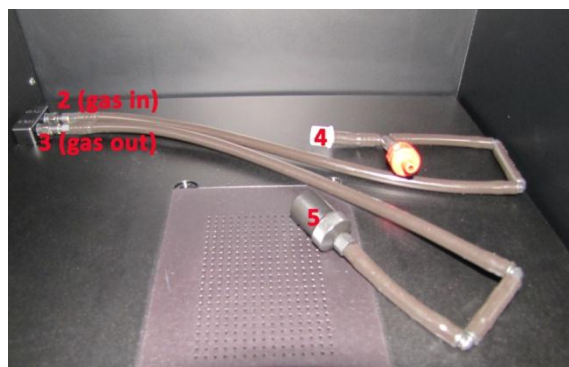
1. prepare the IVIS imaging chamber for connection with the XIC-3 Animal Isolation Chamber
2. how to connect the LEI bench top anesthesia machine to the XIC-3 to maintain the animals under anesthesia
3. how to connect the XIC-3 to the IVIS imaging chamber with attached XGI-8 anesthesia machine.

All **F-Air canisters MUST BE weighed** prior to connecting the XIC-3 isolation chamber and anesthetizing any animals. The F-Air canisters are

1. IVIS chamber/manifold canister located on top of the XGI-8 anesthesia machine
2. The canister for the LEI bench top anesthesia machine and the canister attached to the XIC-3 isolation chamber.
3. The canister attached to the XIC-3 Gas Out tube that should be attached to the back of the IVIS Spectrum after the Gas Out tube (yellow tag) to the XGI-8 has been removed

### PREPARATION OF THE IVIS IMAGING CHAMBER FOR THE XIC-3 ANIMAL ISOLATION CHAMBER

1. Disconnect the IVIS Gas Out Tube (**1**) (yellow tape) from the back of the IVIS Spectrum. This tube connects the IVIS Gas Out to the XGI-8 anesthesia machine.
2. Connect the XIC-3 Gas Out Tube with attached F-Air canister to the Gas Out connection on the back of the IVIS Spectrum. The F-Air canister should be laid on the floor on its side, do not stand it on its end on the floor.
3. Disconnect the five station manifold from the inside of the IVIS imaging chamber.
4. Connect the tubing for assembly with the XIC-3 isolation chamber to the Gas (**2**) and EXH (gas out) (**3**) connectors inside the IVIS imaging chamber. Connect the ends of these two tubes, **4** and **5**, together. Isoflurane gas will not escape into the chamber when these ends are connected.



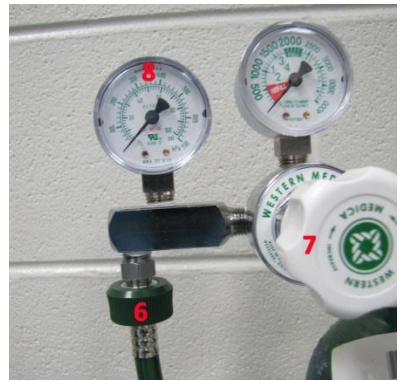
To have oxygen/isoflurane flowing into the IVIS imaging chamber with the above tube assembly, follow the instructions in the quick reference protocol for the XGI-8 anesthesia system up to step 6.

Turn ON the “IVIS Flow on/off” toggle and adjust the flow rate to 1.75 lpm, oxygen should be flowing through the tube connections. Adjust the isoflurane level to 2.5% by turning the dial on the vaporizer. Turn OFF the “IVIS Flow on/off” toggle until the XIC-3 isolation chamber is ready to be connected.

### ASSEMBLY OF THE LEI BENCH TOP ANESTHESIA MACHINE TO THE XIC-3 ISOLATION CHAMBER.

After cleaning out the hood with SporKlenz and preparing a 1% bleach solution, wipe off the LEI bench top anesthesia machine and place it in the hood.

1. Connect the LEI bench top anesthesia machine to the oxygen tank as follows:  
Connect the female fitting on the end of the green hose (6) to the male DISS fitting on the oxygen regulator which is attached to the oxygen tank.  
This can be tightened by hand.



The XIC-3 Animal Isolation Chamber should be connected to the LEI bench top anesthesia machine as illustrated in the picture below.

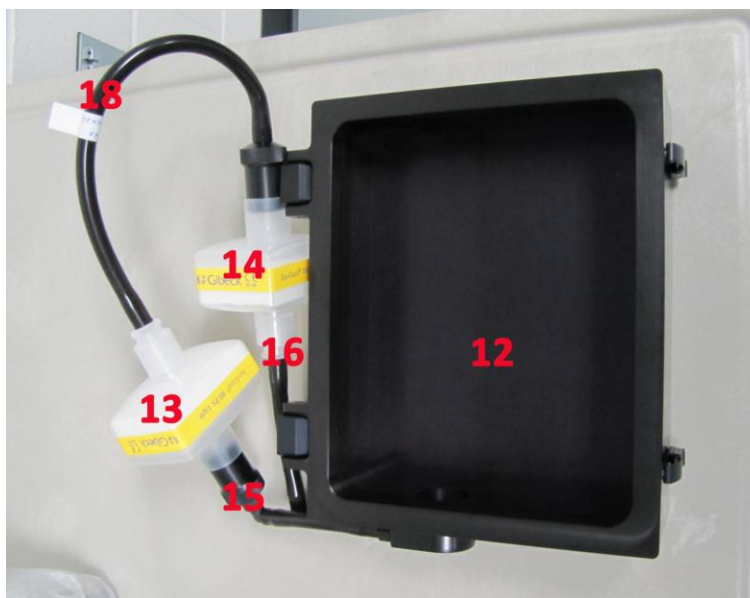


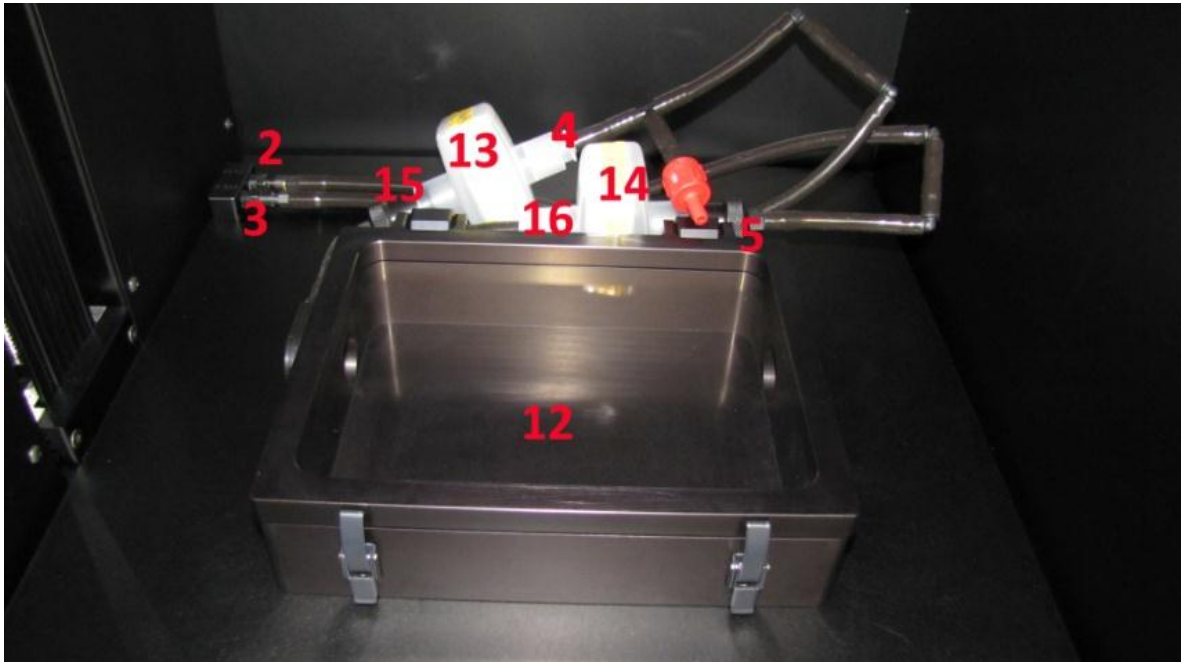
2. The induction chamber (9) is connected at position 2 (10) of the multi-port manifold (11). Wipe out the induction chamber using the cloths from the 1% bleach bath. Place a sterile Wypall cloth in the bottom of the induction chamber.
3. Carefully place the XIC-3 Animal Isolation Chamber (12) in the hood. Be careful not to touch the window of the imaging chamber as you do not want marks or smudges on this surface.
4. Attach the Gibeck Isogard HEPA filters (13 & 14) to the gas in (15) and evacuate/exhaust (16) tubes on the XIC-3 isolation chamber (12).
5. The “gas in” HEPA filter (13) is connected at position 3 (17) of the multi-port manifold on the LEI bench top anesthesia machine
6. The HEPA sealing assembly tube (18) is attached to the “gas out” HEPA filter (14) and to a F-Air canister (19)

With the XIC-3 Animal Isolation Chamber connected to the LEI bench top anesthesia machine the mice can now be anesthetized.

## ANESTHESIA PROTOCOL USING THE LEI BENCH TOP ANESTHESIA MACHINE AND XIC-3 ANIMAL ISOLATION CHAMBER.

1. Check the isoflurane level in site glass (20) of the vaporizer (21). Isoflurane should only be added using the anti-spill bottle adapter while the anesthesia machine and oxygen supply are turned off.
2. Using the wrench, slowly and carefully open the oxygen cylinder. Adjust the pressure to the anesthesia machine by turning the white knob (7) on the regulator. The pressure should be set to 50 psi on the gauge (8).
3. The flow meter (22) on the LEI anesthesia machine should be adjusted to 1 lpm.
4. The blue lever at position 2 (10) on the multi-port manifold (11) should be turned on to allow oxygen to flow into the induction chamber.
5. Place the mice to be anesthetized in the induction chamber (9).
6. Adjust the isoflurane level by pushing down on the black release tab on the dial (23) of the vaporizer and turn the dial to 2.5%.
7. Monitor the decline in activity and change in respiration in the animals in the induction chamber. When they have reached the desired depth of anesthesia turn off the isoflurane to the induction chamber by closing the blue lever at position 2 (10) on the multi-port manifold (11).
8. Quickly transfer the animals to the XIC-3 isolation chamber.
9. Turn on the isoflurane to the XIC-3 isolation chamber by turning the blue lever at position 3 (17) on the multi-port manifold (11).
10. Monitor the mice in the XIC-3 isolation chamber to ensure they are fully anesthetized.
11. Turn on the "IVIS Flow on/off" toggle on the XGI-8 anesthesia machine so isoflurane flows through the tubing in the IVIS imaging chamber.
12. Disconnect the XIC-3 isolation chamber from the LEI bench top anesthesia machine by turning the vaporizer dial (23) to OFF.
13. Disconnect the "gas in" HEPA filter (13) from the tube connected to position 2 (10) of the multi-port manifold (11).
14. Disconnect the HEPA sealing tube assembly (18) from the F-Air canister (19).
15. Quickly connect the HEPA sealing tube assembly (18) to the "gas in" HEPA filter (13). This prevents isoflurane gas escaping while the XIC-3 isolation chamber is brought to the IVIS Spectrum.
16. Take the sealed XIC-3 Isolation chamber to the IVIS Spectrum imaging chamber.





17. Quickly disconnect the HEPA sealing tube (18) from the HEPA filters (13 & 14) and disconnect the tubes in the IVIS imaging chamber (4 & 5)
18. Quickly connect the “gas in” HEPA filter (13) with the “gas in” tube (4) of the IVIS.
19. Quickly connect the “gas out” HEPA filter (14) with the “gas out” tube (5) of the IVIS.
20. Monitor the mice to ensure they are at the correct depth of anesthesia before imaging. Adjust the level of isoflurane if necessary.
21. Once imaging is complete turn off the “IVIS Flow on/off” toggle, turn the dial of the vaporizer on the XGI-8 anesthesia machine to off.
22. Disconnect the XIC-3 isolation chamber from tubing in the IVIS imaging chamber.
23. Connect the HEPA sealing tube (18) to the HEPA filters (13 & 14) on the XIC-3 Isolation Chamber.
24. Place the XIC-3 Isolation Chamber in the biosafety cabinet, carefully remove the animals and return them to their cage.
25. Monitor them to make sure they fully recover from the anesthesia.
26. Once all imaging involving the XIC-3 Isolation Chamber is complete use the wrench to close the valve to the oxygen tank. The pressure gauges on the oxygen tank should both return to zero and the flow meter on the LEI bench top anesthesia machine should also be at zero.
27. Clean out the induction box (9) and remove the LEI bench top anesthesia machine from the biosafety cabinet.
28. The XIC-3 Isolation Chamber should be cleaned according to the protocol provided.
29. Once imaging using the IVIS Spectrum is complete, the oxygen has been turned off and all flow meters read zero, the XIC-3 Gas Out Tube with attached F-Air canister must be removed from the back of the IVIS Spectrum.
30. The IVIS Gas Out Tube (1) (yellow tape) from the XGI-8 anesthesia machine must be re-connected to the back of the IVIS Spectrum.

**REMEMBER TO WEIGH ALL F-AIR CANISTERS AND NOTE THE WEIGHTS ON THE LOG-SHEETS. REPLACE THE F-AIR CANISTERS WHEN THEIR WEIGHT HAS INCREASED BY 50G.**