

Protocol for setup of germ-free isolator

To prepare a germ-free isolator the inside of the isolator must first be cleaned ready for use. At this stage it is not necessary to work in a sterile manner, but all materials should be kept as clean as possible. Please remember that you must always be very careful not to tear the plastic film of the isolators; this will break the seal and compromise sterility when they are in use.

Stages of the procedure

The isolator is to be set up in the following stages

1. Cleaning and checking mountings
2. Leak test
3. Decontamination with 3% peracetic acid
4. Start ventilation

Equipment required

- A. Dustpan and brush
- B. Small bowl of warm water
- C. Stopwatch
- D. Protective mask and clothing to handle peracetic acid (see **Gnoto1005**).
- E. Supply of concentrated peracetic acid.
- F. 250ml Duran bottle with cap.
- G. Gauze swab loaded onto swab holder.
- H. Spray gun and pressure hose.
- I. Spray gun container with 200ml of 3% peracetic acid (freshly made up).
The lid on the container should be tightly screwed down.
- J. Parafilm.

Procedure detailed

1. Remove all materials from the isolator. Use a dustpan and brush to sweep up old sawdust/particulates and then clean the inside of the plastic flexible film with soapy water and a soft cloth.
2. Dry the flexible film and floor of the isolator.
3. Replace the doors of the isolator on both quadralocks.
4. Close or bung all other ports.
5. Start the motor to inflate the isolator to working pressure, and wait until the isolator is completely dry inside.
6. Remove the port for the exhaust air and place a bung tightly in the hole.
7. Allow the isolator to go 20mmHg (15.2mBar) over pressure and then switch off the blower and place a bung in the inflow port.
8. Start a stopwatch and note the pressure at time zero, then take readings at 15 minute intervals, the seal is satisfactory provided that the pressure falls no more than 6mmHg (4.56mBar) after the first hour and 9mmHg (6.84mBar) after the second hour. IF THE PRESSURE FALL IS GREATER THAN THIS YOU NEED TO LOCATE AND REPAIR THE LEAK (SEE SEPARATE OPERATING PROTOCOL).
9. DO NOT remove the bungs at this stage or restart the blower.

10. Put on a protective mask and clothing prior to making up peracetic acid in the next step.
11. Make up 200mls of 3% peracetic acid (CAUTION, PERACETIC ACID IS BIOHAZARDOUS, YOU MUST READ THE SAFETY SHEET AND THE OPERATING PROTOCOL [GNOTO1005] TO MAKE IT UP AND TO USE IT BEFORE DOING THIS).
12. Place 140ml 3% peracetic acid in the reservoir of the spray gun and place the spray gun inside the isolator. Place 10ml of 3% peracetic acid in a 100ml Duran bottle and place inside the isolator.
13. Dip the swab (preloaded onto the swab holder) into the 10ml of 3% peracetic acid and carefully ease out the bungs and wipe them and the in and out ports and all small ports of the isolator. Reseal the bungs and drop the swab into the Duran bottle. Remove the bottle and the holder from the isolator.
14. Attach the pressure hose connection through the sampling port of the outer door of the quadralock, and lock the outer door into position. The inner door should lie inside the isolator. Seal the hose to the sampling port with parafilm
15. Switch on the compressor.
16. Mist all surfaces of the inside of the isolator with the spray gun. Spray over the inner door and the inside surface of the quadralock and into the in port of the isolator (always keep the tip of the spray gun at least 6 inches (or 15 cm) from the opening of the in port).
17. Switch off the compressor to the spray gun.
18. Run the blower on reverse for 15 seconds to suck air back through the in port.
19. Reseal bung over the in port.
20. Disconnect the attachment on the pressure hose and leave the gun inside the middle of the quadralock. Coil the disconnected hose into the middle of the quadralock and replace the inside door.
21. Leave overnight and then remove bungs and restart the isolator blower.