

*STANDARD OPERATING PROCEDURE***Anaerobic Chamber**

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Minimum Personal Protective Equipment Required: Skin and body protection (long pants, closed toed shoes, appropriate gloves)

Risks: Explosions can be triggered if hydrogen concentrations are >4%. Resultant fire and/or pressure wave can cause extreme injury or death.

Explosion, release of toxic gases and/or asphyxiation if compressed gas is rapidly released, frostbite from liquified gases. See *Compressed Gas SOP* for more details.

Special Handling:

- ✓ You may **NOT** use the anaerobic chamber until you have been fully trained by Aifen, Megan, or Tao
- ✓ Remove any rings or watches that might puncture the chamber gloves
- ✓ Plan out experiment and make sure you have all the supplies you need to minimize opening and closing of chamber

Protocol/Procedure:

1. Verify the identity of connected gases before use. Always use a nitrogen-low hydrogen mix from the supplier. Never make your own mixture.
2. Open Gas-mix and Nitrogen cylinder regulators. Check level of gas in each tank, if low (~100psi on **tank pressure** gauge), secure replacement cylinders from stockroom before proceeding.
3. If transfer station is **NOT** “Anaerobic”, cycle transfer station using P9 program.
4. Check last date Stak-Paks were replaced. If more than a week, replace them following instructions below.
5. If waste receptacle is full, or any other “trash” is inside the chamber, move it to transfer station for removal (dispose of this waste accordingly, do not leave it in the room).
6. Place your items inside the transfer station. Close the door, cycle the transfer station using the P9 program.
7. Transfer your items to the chamber, sealing the door afterwards.
8. The hydrogen partial pressure should read **3-3.5%**. If not, manually adjust concentration using controls on the transfer station.
9. Ensure oxygen detector reads **0ppm** before proceeding with anaerobic work.
10. When you have finished working with the chamber, REMOVE ALL YOUR WASTE AND OTHER ITEMS. (Do not leave syringes, needles, pipettes, pipette tips, swabs, wipes, inoculating loops or serum bottles in the bag)

11. Before you leave the room, ensure
 - The transfer station is “Anaerobic”.
 - All cylinder regulators are closed.
 - All waste is removed and disposed accordingly.

Stak-Pack Replacement:

1. The Stak-Paks need to be replaced every week. Please ensure this is done as follows-
2. A duplicate set of Stak-Paks is in the drawer below the chamber.
3. Place these Stak-Paks in the baking tray and then place the tray in the oven @ 75°C for 2 hours.
4. Remove the Stak-Paks, let them cool to room temperature and then place one pair in each fan box.
5. Do not leave freshly re-generated Stak-Paks inside the transfer station for too long, they will absorb H₂ and O₂ creating a vacuum inside the transfer station.
6. Return the expended set of Stak-Packs and backing to the drawer.