

Use of Autoclave

Most buffers and other solutions used in any lab are sterilized before use to prevent bacterial and fungal growth during storage. There are two basic techniques for sterilizing solutions: autoclaving and sterile filtration. Most buffers and other salt solutions are autoclaved, because filtration of large volumes is time-consuming and disposable sterile filters are expensive. However, before autoclaving any solution you should always check whether it contains any heat-labile ingredients. If it does, the heat-labile substance will usually have to be prepared separately, filter-sterilized, and added to the remainder of the solution after autoclaving.

autoclave: most buffers and salt solutions
 undefined bacterial and yeast media

do not autoclave: buffers with detergents (SDS) – they will boil over
 organic solvents (ethanol, acetone, phenol, chloroform)
 heat labile ingredients (vitamins, hormones, antibiotics, proteins)
 HEPES-containing solutions
 DTT- (dithiothreitol) or BME- (beta-mercaptoethanol)

Autoclaves heat their contents to 121 °C, which is 21 °C over the boiling point of water. To prevent the solutions from boiling over/vaporizing, the autoclave chamber is pressurized during this process. The department currently owns two autoclaves that function slightly differently; ask someone to help you if you should forget about the idiosyncrasies of either machine.

Be sure your items are really autoclavable: bottles should be made from borosilicate glass or autoclavable plastic – be sure that any plastic items you put in the autoclave are really autoclavable. Pipet tips, microfuge tubes, and their storage containers generally are, ask about other plastics before you experiment.

1. Leave at least a quarter of the container volume as free space (otherwise your solutions will boil over).
2. Place containers in autoclavable pans to catch liquids from any breaking containers.
3. Make sure all caps are loose and taped on at least on one side (including tinfoil caps)
4. Use autoclave tape (TimeTape) to tape on caps, also put a small bit of autoclave tape on any other items you autoclave. The tape will change color during autoclaving, thus signaling to any future user that the item has really been sterilized.
5. Tighten autoclave doors thoroughly but not so much that you can't get it back open.
6. Select the appropriate program:
 - minimum autoclave time should be 20 min
 - use longer times for larger volumes
 - use the "liquid cycle" for any load containing liquids
 - use the "dry cycle" only if there are no liquids in the load (autoclave will exhaust faster with this program, causing any liquids to boil over)
 - the "baby autoclave" should be set for 40 minutes (20 minutes to heat up to sterilizing temp, 20 min to sterilize), the "big autoclave" figures in heat-up time automatically
7. Make sure the pressure is down completely (check gauge) before opening, this will take about 20 minutes for the baby autoclave after the cycle is through
8. Open the door VERY SLOWLY and STAND BACK unless you want your face scalded
9. Wear heat-resistant gloves to unload the autoclave
10. Put glass bottles on stacked paper towels or potholders, they may crack if put on cold counters
11. CLEAN UP ANY SPILLS, empty any liquids from autoclave pans down the drain, rinse well