

⊘⊘⊘⊘ **Autoclaving**

Autoclaving is a process by which high pressure steam is used to clean and sterilize lab equipment and solutions, as well as break down nutrient rich tissues that could become a biohazard. The steam, which is kept at 121°C, is far above the boiling point of water and is consequently fatal to most microbes and viruses. Despite this, the process has no effect on prions or some strains of archaea capable of surviving higher temperatures.

This protocol is written with specificity for the autoclave machine in room 3152. If a different autoclave is being used, *please update this protocol*.

Note that the methods for glassware sterilization will require some kind of liquid be placed in the bottom of the containers being sterilized-this can keep the container from fracturing in the heat, if composed of glass. In the normal case of sterilizing glassware after washing, this will be distilled water; in the process of making stocks of nutrient broths, this will be LB solution. The solution cannot contain significant amounts of mineralized (“hard”) water, however, as this will cause boiling over.

Compounds

Your favorite aqueous solution not containing hard water

Materials

Washed flask(s)

Aluminum foil

Autoclave tape

Autoclave tray

Thick, heat resistant gloves (NOT LATEX GLOVES!)

You will also need access to an:

Autoclave

Procedure I

1. Fill your **washed flask** with **your favorite aqueous solution**. Fill to around half the volume of the flask.
2. Cut or tear a square of **aluminum foil** larger than the mouth of the flask by 2-3cm on each side.
3. Cover the top of the flask with the foil, then squeeze and press it around all sides of the flask. This should form a “cap” on the flask and keep it sterile.
4. Cut a ~2cm piece of **autoclave tape**. Press it on the top of the aluminum foil.
5. Place all glassware and articles in an **autoclave tray**. Slide the tray into the **autoclave** machine.

6. Follow the directions on the front of the machine to operate it properly. The autoclaving process may take between **50 minutes** and **several hours** depending on the settings.

After the process is complete, remove the tray with **heat resistant gloves** and store the glassware. Liquids within the glassware will be sterile and very, very hot.