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Autoclaving Erlenmeyer Flasks for Sterile Algal Cultures

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1 Works for me dx.doi.org/10.17504/protocols.io.bd2di8a6

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ABSTRACT

This protocol describes autoclaving the Erlenmeyer flasks for sterile microalgae culture. It describes the cleaning and preparation of flasks for autoclaving. In the second part, the protocol describes the method of autoclaving.

EXTERNAL LINK

https://app.labstep.com/sharelink/8251a76e-d779-469b-8d01-e4627440d4b0

GUIDELINES

This protocol describes autoclaving the Erlenmeyer flasks for sterile microalgae culture. It describes the cleaning and preparation of flasks for autoclaving. In the second part, the protocol describes the method for autoclaving.

This protocol is highly specific to the type of autoclave used locally and does not translate widely. Use whichever protocol is locally approved to autoclave the culture flasks. If no autoclave is available, use sterile single-use tissue culture flasks instead.

This protocol will take about 2 hours, with an overnight wait for the flasks to dry.

MATERIALS TEXT

Culture Flask Preparation Materials

Borosilicate Glass Narrow Neck Erlenmeyer Flasks (100 ml)
by Fisher Scientific
Catalog #: 15409103

Any other similar flask made of glass can be substituted.

Cotton Wool
by Fisher Scientific
Catalog #: 12356477

Only use clean pure cotton wool. Pure personal cosmetics cotton wool can be substituted as long as it is not dyed, scented, or oiled.

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Aluminum Foil 24 µm Thick
by Thermo Scientific
Catalog #: 11779408

Use at least 24 µm thick foil, if possible. Standard kitchen foil is too thin and not recommended, as it tears easily. If kitchen foil is the only one available, fold it over to double or triple the thickness.

- Dish Washing Liquid
- Flask Cleaning Brush
- Dish Drying Rack
- Deionized Water or distilled water or at least clean soft water not causing limescale

Autoclaving Materials

8-Liter Autoclave
Portable Stainless Steel Pressure Steam Sterilizer

China XFS-D-8L
Voltage: 220 V (AC)
Power: 1.2 kW
Working Medium: Steam
Design Pressure: 0.17 MPa
Working Temperature: 129 °C
Frequency: 50 Hz
Useful Life: 5 Years
Delivery Date: 3. Oct, 2019

This is a low end autoclave. Use the most approriate autoclave available in your settings.

Plug-In Electronic Boost Timer

Timeguard TGBT6
Maximum Load: 3 kW
Maximum Current: 13 Amp (Resistive)
ON Time Increments: ¼, ½, 1, and 2 hours

The timer used here is for UK sockets only. The timer is used as a backup to turn off the autoclave in case it is forgotten unattended. Other timers will be required in other world regions. The timer must have sufficient load power rating (3 kW) and support a 30 minute timeout, which will turn off the autoclave automatically.

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- Autoclave Tape
- Lab Jack or other heat proof support

SAFETY WARNINGS

Cleaning described in this protocol uses dish washing liquid. Protect skin and eyes by using appropriate personal protection equipment.

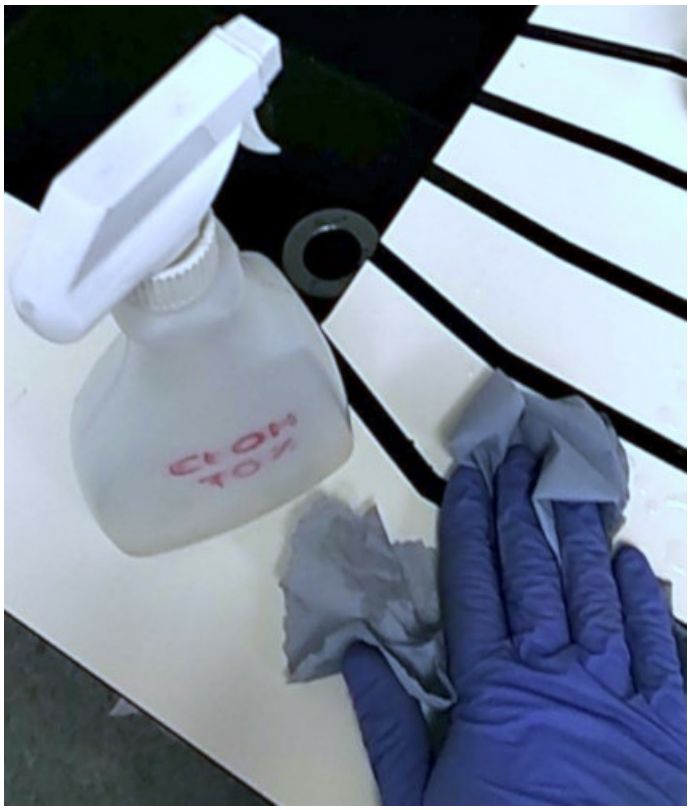
Autoclaving comes with numerous risks. Installation and operation of autoclaves must follow manufacturer's instructions. This document by no means covers various aspects of safe operation of autoclaves and must not be considered as such.
Ensure all safety risk assessments are established. Put risk mitigation strategies in place. Ensure all equipment is safe to use and regularly tested. Allow only trained staff understanding the risks operate the autoclave. The risks include high temperature, high pressure, super-critically heated liquid, steam generation and release, electrical and fire hazards. Improper autoclave operation or equipment failure might result in insufficient sterilization and thus create potential biological hazard and contamination of cell cultures. We take no liability for any accidents or experimental outcomes resulting from following this and other protocols.

BEFORE STARTING

Ensure that you have an autoclave available and all that proceedures, training, and risk assessments for its safe operation are in place.

Cleaning Culture Flasks

1 Wash the dish washing area with 70 % denatured ethanol.



Wipe down dish washing area

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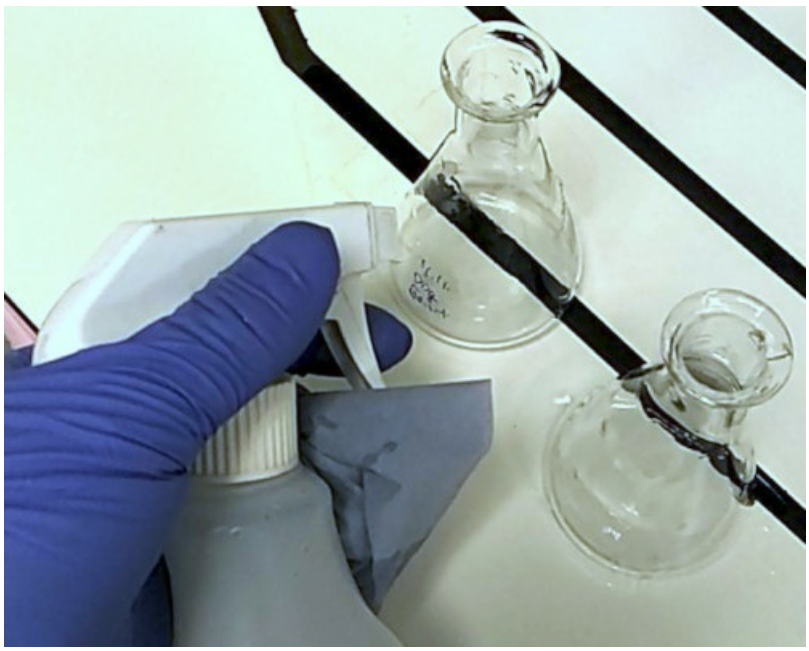
2 Wash away all permanent marker labels and writing from the Erlenmeyer flasks using 70 % denatured ethanol.



Wash off permanent marker with 70 % ethanol.

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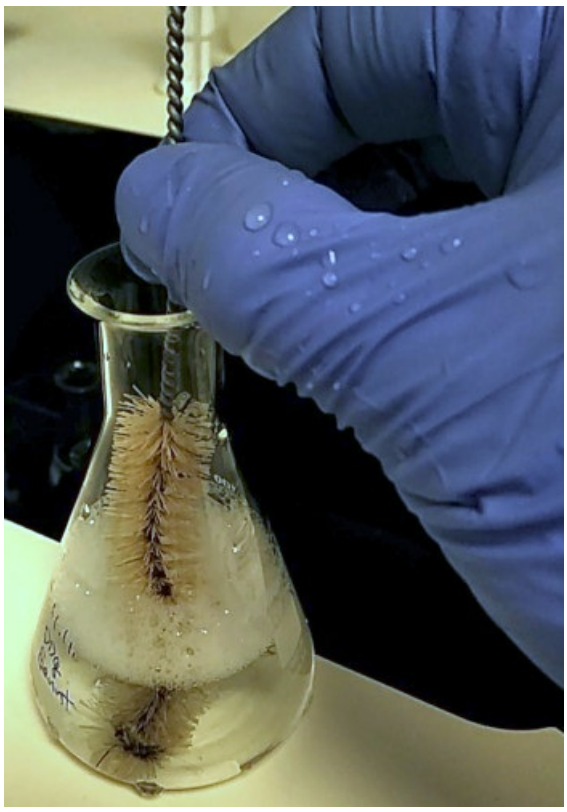
3 Wash used flasks with dish-washing liquid using vial cleaning brush. Scrub off any dried deposits of algae.



Use dish-washing detergent and vial cleaning blush to scrub the inside of the flask.

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4 Thoroughly rinse many times with copious amounts of hot and cold water to remove all detergent.

Fill up each flask with hot wter and empty it at least 10 times to remove all detergent.

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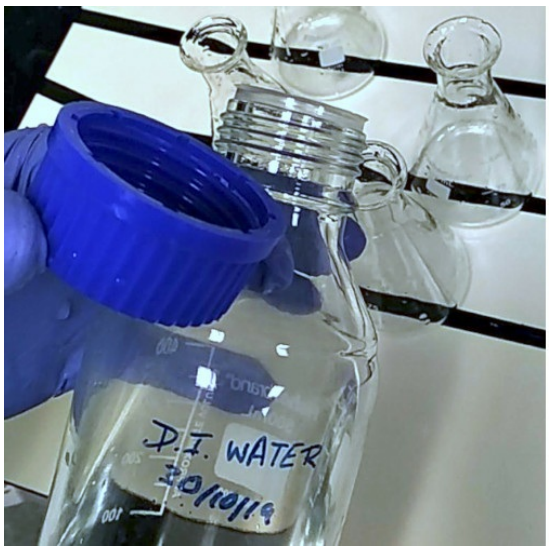
5 Rinse off tap water from within the flask with a small amount of deionized water, swishing around the entire inside of the flasks. Repeat, if possible.

This step might not be essential in areas with soft water supply. However, in hard water areas, using only tap water will lead to accumulation of limescale attracting cell debris that will get progressively harder to wash away without the use of acids.



Pour a dash of deionized water into each flask (left). Swish it around and empty it by abrupt turning upside down (right), to rinse the entire inside of the flask with the deionized water. Repeat for improved rinsing.

6 Let the flasks dry on a drying rack.

Flasks drying on a wall-mounted rack.

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Preparing Flasks for Autoclaving

7 Once completely dry, seal each flask mouth with a cotton wool roll or ball.

Plug the flask opening with clean cotton wool.

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8 Cut about 10 cm × 10 cm (4" × 4") piece of aluminium foil for each flask.

If the aluminium foil is thinner than 24 µm, such as standard kitchen aluminium foil, make pieces larger and fold them over to create two or three ply thick pieces to seal the flasks with.

Five alimunium foil pieces ready to wrap around the openings of five flasks plugged with cotton wool.

9 Seal the flask opening with the aluminium foil covering the cotton wool.

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Gently wrap the aluminium foil over the opening of the flask. Do not wrap it too tightly. It should not be too loose fall off, but it should not be to tight to make later removal difficult.

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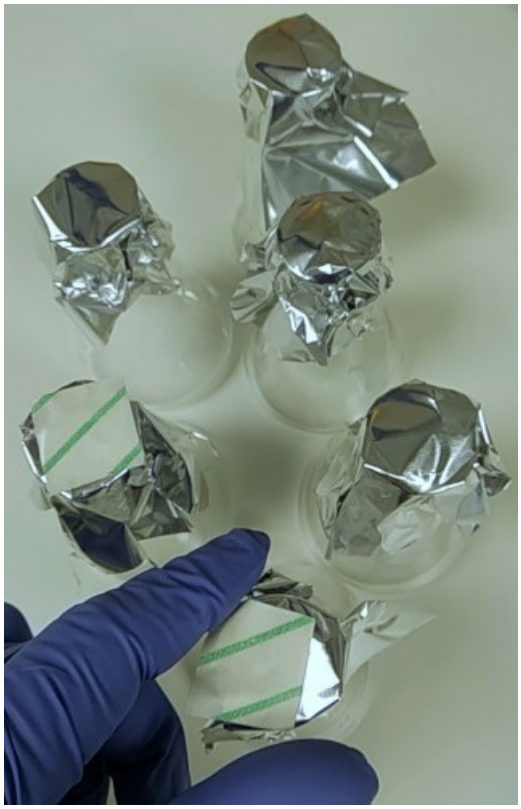
10 Cut a small piece of autoclave tape and stick it to the top of the aluminium foil. Do not tighten the aluminium foil with the autoclave tape, as it would make it hard to remove later.



Stick a small patch of autoclave tape over the aluminium foil wrapping around the flask opening.

Preparing the Autoclave

11 The procedure below is specific to the autoclave and the conditions in our lab. This protocol is not universally transferable and it must be redesigned for local equipment, conditions, and safety regulations.

Use this protocol only as a guidance to develop your own protocol.

Beware, this type of autoclave does not have a vacuum pump. It is therefore not suited for autoclaving bags of waste, sealed vessels, pipette tip boxes, and any containers into which the steam will have problem penetrating efficiently.

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12 Prepare all the autoclave equipment as listed in the Materials section above.

Autoclave parts and a sturdy lab jack laid out on a tidy lab bench.

13 Prepare a tidy space on solid desk.

Place a strong lab jack or other heat-resistant support away from the edge of the desk.

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Sturdy lab jack, which serves as a heat-resistant support for the autoclave.

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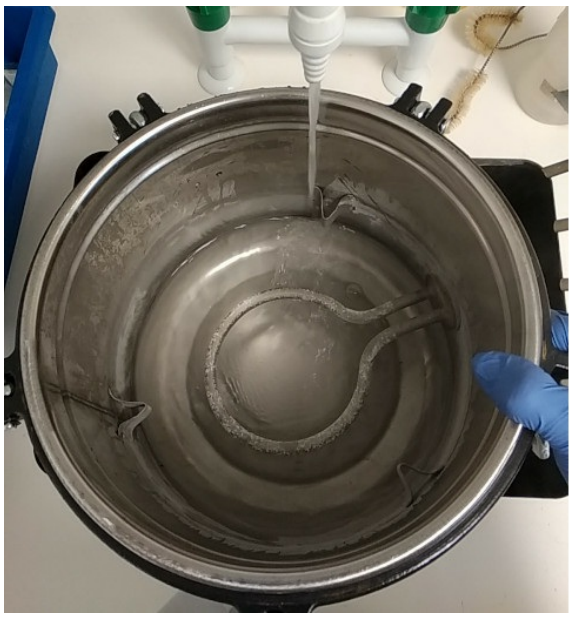
14 Pour hot tap water into the autoclave up to 1 cm (½") below the heating spiral seal, as in the image below.

Ensure no water reaches the electrical connector on the outside of the autoclave while filling it up.

You can also use cold tap water, but then the autoclaving will take longer to finish.



Fill up the autoclave with hot tap water (left). The entire heating spiral must be submerged and the water level should be about 1 cm below the heating spiral seal.

15 Place the autoclave on the heat-resistant support.

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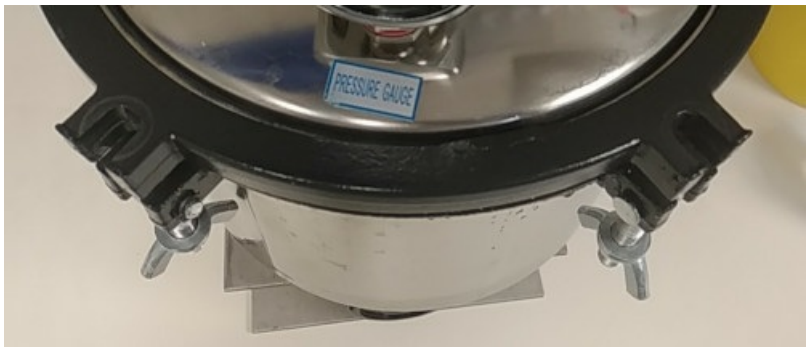
16 Insert the autoclaving container and load it with the prepared conical flasks. The autoclave will fit nine flasks comfortably, and will not fit more than ten.

Autoclave loaded with six conical flasks ready to be sealed and turned on.

Sealing the Autoclave

17 Place the lid over the autoclave, aligning the fixing loops with the screws on the autoclave body.



Autoclave lid aligned with the fixing screws of the autoclave.

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18 **Orient the autoclave so that the pressure release valves are pointing towards the wall, and definitely not into the room.**

The released steam could injure someone if the valve point in the direction where people may happen to be.

Steam release valves on the autocalve lid are pointing towards the room away from places where people may happen to be.

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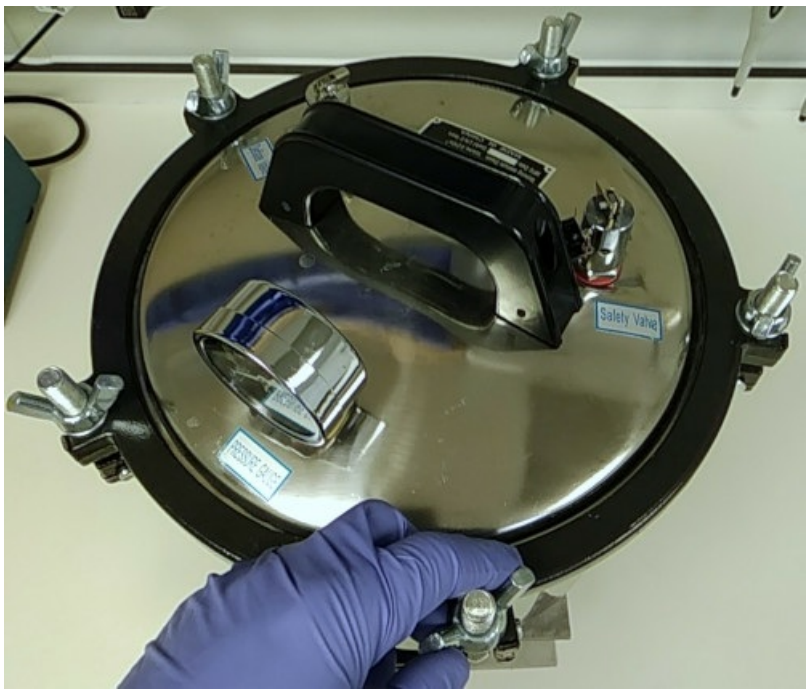
19 Screw all five sealing wing nuts gently.



Gently tightening the wing nuts holding the autoclave lid.

20 Start tightening the wing nuts in small steps at a time. Create and observe a tightening step order, in which neighboring wing nuts are never tightened after one another. For clarity, wing nuts are labeled A–E, in the picture below. The tightening sequence could be, for instance, A–C–E–B–D–A–C–E–B–D–…

Keep tightening the wing nuts, repeating the same sequence, until all wing nuts are fastened hand tight. The autoclave comes with a tightening spanner, but this was never required.

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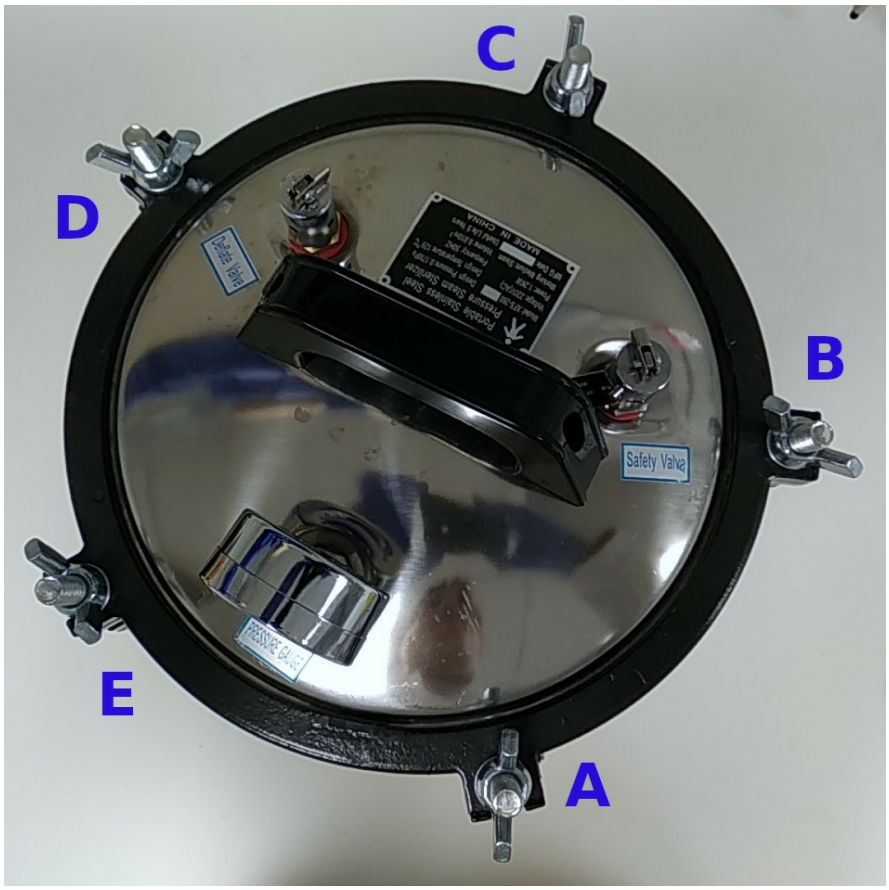

Each wingnut has been asigned a unique letter to explain the tightening sequence. The tightening sequence is such that neighboring wing nuts are never tightened one after another.

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Operating the Autoclave

21 Plug the power supply lead into the autoclave.

Make sure the lead and the autoclave electrical connector are completely dry before this step.

If the electrical connections are wet, let them dry out over a long period of time and/or consult H&S officer to agree the best ways to proceed.

Plug the mains lead into the autoclave.

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22 Plug the power plug leading from the autoclave into the Countdown Timer.

Safety count-down timer tha would switch off the autoclave if left unattended

23 Plug the Countdown Timer into a mains socket.

24 Turn on the mains socket using the switch provided.

Pressing the delay button on the Countdown Timer twice, to set it to ½ hour.

Two blue lights will light up, meaning the autoclave is on and the 30-minute countdown has started.

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Set the countdown timer to 30 minutes.

25 Keep attending to the autoclave while it is heating up and the pressure is building up.

Observe the pressure gauge regularly.

Pressure in the autoclave will gradually rise as the temperature inside increases.

The autoclave takes about 15 minutes to heat up to the operating temperature, when starting with hot tap water. It will take longer with cold water.

26 **Once the autoclave reaches a pressure of 0.18–0.19MPa, the safety valve will release the steam. This lowers the pressure (and temperature) inside the autoclave sufficiently for the safety valve to close again and allow the autoclave to start heating itself up.**

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26.1 The autoclave is designed to cycle between the process of heating up and releasing the pressure (and temperature) as long as the power remains on.

This leads to cycling of temperature and pressure inside the autoclave, helping the penetration of steam into any closed vessels.

The release of steam also warms up the room. The released steam may cause damage to skin or eyes if they get exposed. Large buildup of steam in the room may trigger the fire alarm. To minimize risk of the above events, the autoclave is manually turned on and off, as described below.

27 Mark the time, when the autoclave reaches pressure of **0.18 MPa** or releases the steam for the first time.

Keep autoclaving for 15–20 minutes after this pressure has been reached.

28 Observe the pressure gauge. When it shows pressure of **0.18 MPa**, turn the power **OFF** at the mains socket.

Do not let the pressure exceed 0.18 MPa.

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28.1 Switch off the autoclave at the power socket to prevent the safety valve from releasing the steam.

Switch off the autoclave at the socket when the pressure reaches 0.18 MPa.

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29 Keep observing the pressure gauge until the pressure drops down to **0.15 MPa**.

Wait for the pressure to slowly drop down to 0.15 MPa or below.

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29.1 This will take about **2:10 minutes**. At that point, turn the power **ON** at the mains socket.

Turn the autoclave back on at the socket when the pressure drops to 0.15 MPa or below.

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29.2 Return to the previous step of observing the pressure gauge and waiting until the pressure raises back to **0.18 MPa**. This takes about **45 seconds**. Turn **OFF** the switch at the power socket again, once the pressure is reached.

Keep to autoclave powered until the pressure returns back to 0.18 MPa.

30 Keep repeating the two steps above until 15–20 minutes pass since the pressure was first reached, or the Countdown Timer runs out and turns itself off. It takes five to seven ON-OFF cycles.

31 Switch off the power at the socket for the final time.

Unplug the Countdown Timer from the socket.

Do not temper with the autoclave until it cools down.

Unloading and Preparing the Autoclave for Storage

32 **Keep the autoclave standing and wait for it to cool down and depressurize itself, ideally overnight.**

You could use the pressure release valve to lower down the temperature rapidly. *However, this is potentially dangerous and is not advisable. It requires physically tempering with the hot autoclave full of hot water and will inevitably release large amounts of hot steam.*

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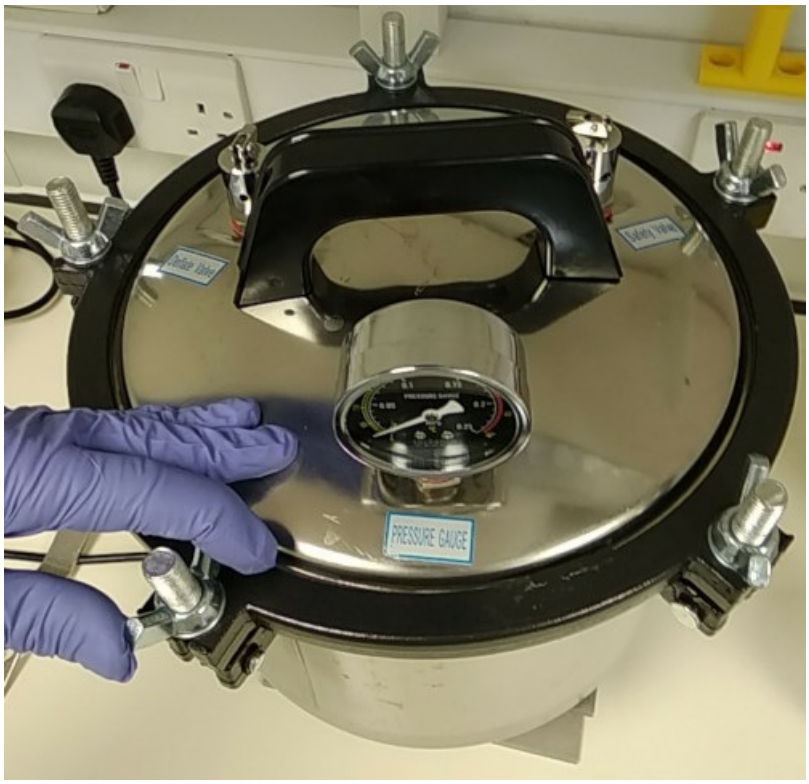
33 Once the autoclave has cooled down to the room temperature, loosen the nuts. Use supplied spanner if they are too tight to loosen them by hand.

Sometimes the autoclave lid is sealed by the underpressure formed inside. Pry it open using the supplied spanner.



Loosen the nuts on the autoclave lid.

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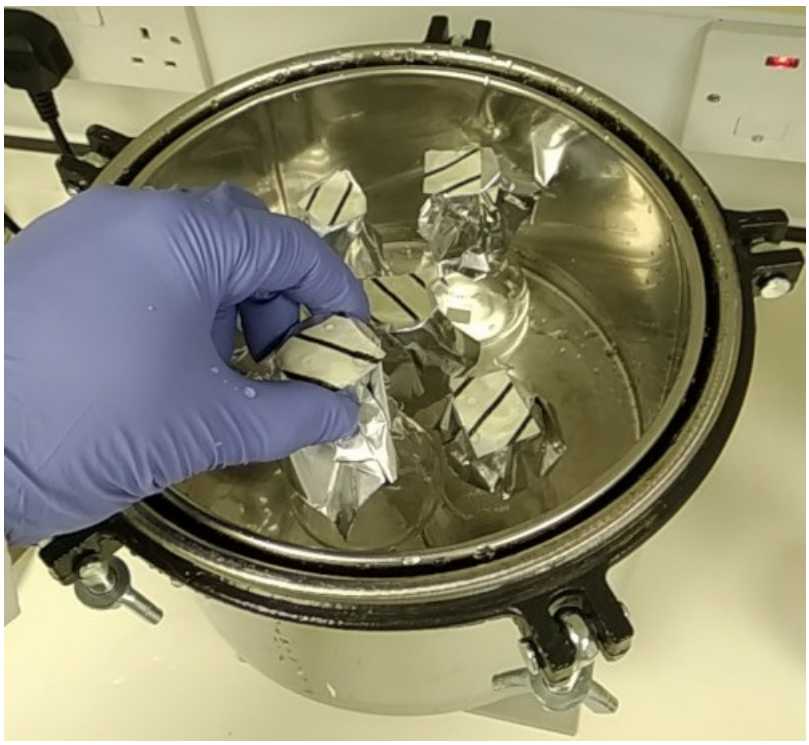
34 Remove the lid and the content.



Unload the autoclave.

35 Empty the autoclave and let it dry before storage.

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35.1

Pour the water out of the autoclave.

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35.2

Let the autoclave dry out before storage. Dry the lid stand facing upside down to let the water trapped in the seal flow out.

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