



# HOW TO AUTOCLAVE GLASS LABORATORY BOTTLES

An autoclave is a specialized piece of equipment designed to provide a physical method for disinfection and sterilization. The process is achieved using a combination of steam, high pressure, and time. Steam is remarkably effective at transferring thermal energy (compared to dry air) and can sterilize a load at 121 °C in just 15 minutes.

The majority of laboratory glass bottles are suitable for autoclaving. However, it is important to take note of some key differences between the types of glass products used in the lab as well as best practice methods when carrying out an autoclave cycle.

In this article we answer common questions with advice that will enable you to autoclave glass laboratory bottles safely.

## 1. Is it safe to autoclave all types of laboratory glass bottles?

Before autoclaving lab bottles, it's important to consider the suitability of different glass and bottle types. Borosilicate glass bottles are safe to autoclave as the material has a very good resistance to thermal shock. There is a much higher risk of breakage when autoclaving laboratory bottles made from soda lime or flint glass and while it remains possible to autoclave bottles of this type, the material doesn't possess the same safety margin.

Plastic coated laboratory bottles can also be autoclaved safely, however, the coating material is subject to hydrolysis by steam during the autoclave process, meaning they are typically able to withstand fewer autoclave cycles than uncoated glass bottles.

You should not typically fill glass containers beyond 75 per cent of the nominal capacity, as this allows for liquid expansion, and possible foaming, preventing overflow. However, the safe filling capacity also depends on the nature of the liquid. Glass bottles containing water or aqueous buffers may be filled to the container nominal fill-line. Whereas bottles used to sterilize agar-based media should only be filled to 50% of the container capacity.

## 2. How should laboratory bottles be loaded into the autoclave?

Firstly, when operating an autoclave, it is advisable to wear personal protective equipment. Typically, this should include full eye/face protection, closed-toe shoes, and heat-resistant gloves to remove items, especially hot glassware.

Avoid over-stacking the autoclave to allow steam to circulate for an effective sterilization process. It is also advisable to ensure bottles have been cleaned before placing them into an autoclave. This will prevent any contaminant substances from baking onto the walls of the glass bottle.

Large heavy bottles, such as 20 L bottles, should also have a small amount of distilled water (WFI or purified) placed inside them to help generate steam when they are being sterilized dry (empty) in an autoclave.

### 3. How should glass bottles with screw caps be steam sterilized?

When sterilizing or autoclaving bottles, the screw cap must be loosely fitted with a maximum of one turn applied. In a closed vessel, the contents will expand causing a large pressure difference (over 4 bar absolute) which may result in an explosive failure. In addition, caps that are not sufficiently unscrewed can create a vacuum in the bottle during the cooling phase. If bottles are not properly vented, the liquid inside cannot boil during the re-cooling phase and thus will remain superheated for an extended time. Never seal or move containers of superheated liquids. If the cap is left loose (1/2 turn) then there is no pressure differential between the steam inside the autoclave and the inside of the bottle.

Bottles may be safely autoclaved with sealed caps if the steam autoclave has support pressure compensation.

In conventional autoclaves (without pressure compensation), a membrane venting cap is a safer and convenient option. A two-way pressure equalization takes place through the 0.2 micron ePTFE membrane, while enabling the cap to remain tightly closed to reduce the risk of contamination after the autoclave process is complete. Membrane venting caps should not be used with bottles larger than 3500 ml.

For a GMP operations, medical applications, or for bottles that are 5 Liters or larger, the use of a single port connector cap fitted with a sterile venting filter would be a more suitable option than the membrane caps. For example, a single-port GL 45 stainless steel cap can be used with a validated pharma-grade sterile-air 0.2-micron 50mm disk or capsule filter. This option is especially recommended for larger bottles over 2 Liters.

### 4. Should I cover the top of the bottles with aluminum foil before autoclaving?

This is a common practice in many research laboratories but putting foil over the mouths of glass containers decreases the likelihood of those items being properly sterilized to begin with. The foil must be applied loose to allow steam penetration and then offers no protection against contamination after sterilization.

### 5. How long should the autoclave cycle last?

Steam sterilization is recommended at + 121, 126 or + 134 °C,

depending on application. For guidance, the British Standard BS EN 285:1997 8.3.1 states: "The holding time shall be not less than 15 min, 10 min and 3 min for sterilization temperatures of 121 °C, 126 °C and 134 °C respectively."

It is good practice to use either a biological or chemical indicator to confirm that the autoclave is working properly. If either indicator fails, the autoclave must be examined to identify and correct the problem. It is also critical to re-autoclave the load once the issue is addressed to ensure sterility.

Autoclave tape can also be placed on items, with marks on the tape becoming visible once critical temperature is reached. However, unlike other methods such as biological indicators, autoclave tape does not provide confirmation of sterilization. Instead, it can be used to identify autoclaved vs. non-autoclaved items.

Finally, when unloading the autoclave, ensure you step aside to keep your face and body clear of the door and steam. It is also advisable to open the door and wait ten minutes to allow items to cool if possible. Always allow autoclaved materials to cool down to room temperature before transporting.

### 6. How often can laboratory bottles be autoclaved?

The number of autoclave cycles that a product can be subjected to depends on the type of glass bottle being autoclaved, with soda lime bottles and plastic-coated bottles able to withstand fewer autoclave cycles than uncoated borosilicate 3.3 laboratory bottles.

Despite borosilicate 3.3's excellent resistance to thermal shock, these bottles can be damaged and become unsuitable for autoclaving over time. This is because most of the tensile strength of borosilicate glass resides in the condition of its surface and if the glass is scratched, even sub-microscopically, its strength is greatly reduced. So, while bottles with an unscratched surface will safely survive repeated pressure cycling, older bottles may not.

Unfortunately, it is impossible to predict when an older bottle might fail. DWK Life Sciences therefore recommends the replacement of articles on a routine basis, and that each article should be thoroughly visually inspected prior to use, for any signs of scratches, chips, or other defects. If found, the product should be removed from service and discarded immediately.



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