

FAQs - FILTRATION

What membrane filter pore size should I use for sterilization?

Regardless of the membrane filter material, always use a 0.2 µm pore size membrane filter as the final filter for any sterilizing filtration. The 0.2 µm membranes used in Thermo Scientific™ Nalgene™ filters have been tested and shown to retain 100% of a challenge of 1×10^7 cfu/cm² *Brevundimonas diminuta* bacteria.

What is the pore size of the glass fiber prefilter?

Fiber prefilters do not actually have an absolute pore size rating because they are depth filters. We rate our prefilters with a relative retention rating or nominal pore size of 1.0 to 1.2 µm. This indicates that the glass fiber filter will retain approximately 80% of all particles larger than 1.2 µm. They are designed to capture larger size particles for general purpose prefiltration applications.

Definition - Absolute retention: A degree of filtration that guarantees 100% removal of suspended solids over a specified size found in the filtrate, e.g. an absolute rated 0.2 µm membrane will remove 100% of all articles equal to or larger than 0.2 µm diameter.

What is the neck size of Nalgene Filter Receiver Bottles? What neck size Nalgene bottle top filters works with Nalgene Filter Receiver bottles?

Nalgene Filter Receivers Bottles have a 45mm diameter neck. Nalgene bottle top filters are available with 45mm neck which fits Nalgene Receiver Bottles or 45 GL neck glass vacuum bottles, and 33mm neck which fits 33-430 neck glass vacuum bottles. Be sure to order Nalgene bottle top filters with 45mm neck if you want to use them with Nalgene filter receiver bottles. If you use glass vacuum bottles, make sure you verify the neck size of the bottle you use to make sure you order Nalgene bottle top filters with a neck size that matches your bottle.

Which Rapid-Flow™ membrane should I use to filter cell culture media or serum?

You have choices for filtering cell culture media or serum: PES or SFCA are preferred.

PES (polyethersulfone), especially the 0.2 µm asymmetric PES in Thermo Scientific™ Nalgene™ Rapid-Flow™ filters is the first choice for sterile filtration of culture media or serum. PES is fast flowing, and has very low protein binding and extractables. If you are want to protect your cultures from accidental contamination by *Mycoplasma sp.*, use Nalgene Rapid-Flow filters with 0.1 µm PES membranes.

SFCA (Surfactant-Free Cellulose Acetate) is also a good choice, especially if you now use filters with CA (Cellulose Acetate) membrane. SFCA contains none of the wetting agents found in standard CA. SFCA also has lower protein binding than standard CA. For some highly proteinaceous fluids (e.g. 100% Adult Animal Sera or Plasma), SFCA may flow faster than PES.

NYLON can also be used but is not recommended if there is serum or protein present in the media.

Which Rapid-Flow filter membrane should I use to filter buffers?

CN (Cellulose Nitrate) membranes are ideal for filtering and clarifying buffers and other aqueous solutions when protein binding is not a concern. These membranes have relatively high protein binding, so are usually not ideal for culture media. Note that Nalgene CN membranes are Triton®-X free.

For cell culture buffers, or if protein binding is a concern, PES should be used.

Which Rapid-Flow filter membrane should I use to filter a solution containing 10% ethanol?

Use Nylon. Nylon membranes are alcohol resistant. They also have low extractables, but are relatively high protein binding so may not be the best choice for culture media.

If protein binding is a concern, use SFCA.

Which Rapid-Flow filter membrane should I use to de-gas acrylamide electrophoresis gels?

The best membrane to use for de-gassing acrylamide gels is 0.8 µm CN (Cellulose Nitrate). The pore size and material allow rapid filtration and removal of gas bubbles. Nalgene Rapid-Flow filter units with 0.8 µm CN membranes are available in 150mL, 250mL, 500mL and 1000mL capacity sizes.

What membranes are in Nalgene Rapid-Flow filters and which one should I use?

Nalgene Rapid-Flow filter units and bottle top filters are available with four different membranes. The type you use depends on why you are filtering in the first place.

PES (polyethersulfone with 0.1 µm, 0.2 µm and 0.45 µm pore sizes). PES is the first choice for sterile filtration of cell culture media and serum. It is fast flowing, has low protein binding and low extractables.

SFCA (Surfactant-Free Cellulose Acetate with 0.2 µm and 0.45 µm pore sizes). SFCA is also a good media and serum filter. It is much cleaner (lower extractables) than CA membranes because it requires no wetting agent, but has slightly higher extractables and binding than does PES. Only Nalgene has SFCA in filter units and bottle top filters

CN (Cellulose Nitrate with 0.2 µm, 0.45 µm and 0.8 µm pore sizes). CN is the standard membrane for general filtration of buffers and solutions. Nalgene CN is Triton®-free. It is fast flowing but has high protein binding and higher extractables than PES, SFCA or Nylon.

NYLON (with 0.2 µm and 0.45 µm pore sizes). Nylon is a tough membrane and has very low extractables; it is naturally hydrophilic. It has excellent alcohol resistance.

If you want to use only one membrane, start with PES. It is the membrane most likely to meet most or all of our needs.

What membranes are in Nalgene analytical filters and which one should I use?

The membranes in Nalgene analytical filter units and funnels are CN (Cellulose Nitrate – a mixed cellulose ester with a predominance of cellulose nitrate material); 0.2 µm for trapping small bacteria and particles, or 0.45 µm pore size for standard microbial QC work. All Nalgene analytical filter CN membranes are certified for water quality work, meeting standards of APHA, EPA, and U.S. Safe Drinking Water Act, and all Nalgene analytical filter funnels and filter units pass ISO7704 testing. These filters are ideal for microbiological QC testing of water, food and beverage, raw materials and finished product. The 0.2 µm are plain white while the 0.45 µm are gridded for easier colony or particle counting. Filter funnels are available with gray membranes that turn black when wet for counting light colored or white microorganisms.

What membranes are in Nalgene syringe filters and which one should I use?

Step 1 is choosing the membrane filter type that is chemically compatibility with your solution: Is it organic or aqueous?

Nylon and PTFE (Teflon) membranes have broad chemical compatibility and can be used to filter most HPLC and GC fluids/solvents. PTFE can also be used to filter air or gases. Nylon is good for DMSO.

PES and CA/SFCA membranes are compatible with non-alcoholic aqueous solutions and are most often used for sterile filtration of culture media, and immunological samples.

Glass-fiber prefilters, or combination filters with prefilter and SFCA membrane are used to filter highly viscous solutions or samples with high particle loads.

Step 2 is determining pore size: What are you trying to accomplish by filtration?

Use 0.2 µm membranes if you want to produce a sterile solution (membranes tested with *Brevundimonas diminuta*) free of all free-living bacteria, or if you need to remove particles equal to or larger than 0.2 µm diameter.

Use 0.45 µm membranes if you want to remove most bacteria (membranes tested with *Serratia marcescens*) or particles equal to or larger than 0.45 µm diameter.

Use 0.8 µm membranes if you want to remove yeast, fungi and fungal spores (membranes tested with *Saccharomyces cerevisiae*) or particles equal to or larger than 0.8 µm diameter.

Step 3 is determining what volume of fluid you need to filter.

Nalgene 4 mm diameter syringe filters (CA, Nylon) are designed to filter 0.5-1.0 ml.

Nalgene 13 mm diameter syringe filters (PES, PTFE) are designed to filter 2-10 ml.

Nalgene 25 mm diameter syringe filters (PES, PTFE, Nylon, SFCA, CA, and glass fiber prefilter) are designed to filter 10-100 ml (up to 200 ml with prefilter).

Nalgene 50 mm diameter inline/syringe filters (PTFE) are designed to filter 0.2-5 L of liquids (larger volumes of air and gases when used as a vent filter)

Can I retrieve the membrane from Nalgene Rapid-Flow filters?

The membrane in Rapid-Flow filters is not intended to be easily removed. Rapid-Flow filters are designed to filter and collect clean or sterile fluids (depending on pore size). To ensure membrane fluid path integrity the membranes are welded in their housing. They are not designed to be removed. If you must remove the membrane, carefully cut around the perimeter of the membrane with a clean scalpel and remove the membrane with forceps.

Note that Nalgene analytical filter units and filter funnels and Nalgene reusable filter holders with receiver and reusable bottle top filters are designed for easy retrieval of membranes, and are recommended if you desire to do this.

What Nalgene filter product should I use to analyze the bacterial or particle count/content of a fluid?

Nalgene disposable analytical filter units and filter funnels are designed for the recovery of particles and microorganisms, and for growth of microorganisms for research and QC testing. They are available with 0.2 μm or 0.45 μm membranes. These membranes are certified to meet EPA requirements for water quality work, and pass ISO7704 testing.

Nalgene reusable filter holders with receiver and reusable bottle top filters can also be used to analyze bacterial or microbial analysis. Since they are reusable and autoclavable, they also reduce the waste stream.

What is the difference between a 0.2 μm membrane and a 0.22 μm membrane?

The difference is due to different pore size measuring methods and the interpretation of those results. In use, the performance of 0.2 and 0.22 μm filter membranes is identical: both are considered "sterilizing" membranes if they are shown to remove a challenge of 1×10^7 CFU/cm² of *Brevundimonas diminuta* under specific test conditions.

What pore size should I use?

The pore size needed for a fluid filtration application depends on the goal of that particular filtration. What do you want to eliminate from the filtrate, or alternatively what do you want to capture on the membrane for analysis? 0.2 μm membranes are typically used to remove or capture bacteria and are considered sterilizing if they have been shown to retain a challenge of 1×10^7 cfu/cm² *Brevundimonas diminuta* bacteria. 0.45 μm membranes are used to remove larger bacteria or particles and are often used in water quality QC testing. 0.45 μm membranes are tested for their ability to remove 1×10^5 CFU/cm² of *Serratia marcescens*. These are the pore sizes most common in Thermo Scientific Nalgene filtration products. There are also two additional pore sizes offered in some products. 0.1 μm membranes are designed to protect against inadvertent *Mycoplasma* contamination and are tested to remove 1×10^7 CFU/cm² of *Acholeplasma laidlawii*. And 0.8 μm is capable of removing a challenge of 1×10^5 CFU/cm²

Saccharomyces cerevisiae and is thus suitable for use with yeasts, fungi, fungal spores and larger particles. It can also be used to de-gas acrylamide gels for electrophoresis.

Pore Size µm	Challenge Organism	Challenge Concentration cfu/cm ²
0.1	<i>Acholeplasma laidlawii</i>	1 X 10 ⁷
0.2	<i>Brevundimonas diminuta</i>	1 X 10 ⁷
0.45	<i>Serratia marcescens</i>	1 x 10 ⁵
0.8	<i>Saccharomyces cerevisiae</i>	1 x 10 ⁵

What total volume can actually be filtered through any given bottle top filter?

This really depends on the solution itself and how quickly it stops/clogs the membrane. A relatively clear solution that does not contain a high number of particles or large amounts of protein will be easier to filter and therefore allow a greater volume to pass through the membrane effectively. On the other hand, a solution with a high particle count may clog the membrane after only a few milliliters pass through. With cell culture media, the amount of serum or protein can have an impact of flow rate and throughput. Bottle top filter capacity (150, 250, 500, 1000 mL) is a general guide to the amount of fluid that can, on average, be filtered.

Throughput of a membrane can be extended by using a prefilter. Prefilters trap most large particles before they reach the active membrane itself. We recommend the use of a prefilter for any solution suspected to contain a significant level of particulates.

Can Nalgene bottle top filters be used with any glass vacuum bottles?

Nalgene bottle top filters are designated as having 33-430 or 45GL neck diameters, and are designed to interface with these threaded neck finishes on glass media/vacuum bottles (or with Nalgene receiver bottles, that have 45GL neck finish). Therefore these are the only sizes of glass vacuum bottles to use. Any bottles used for vacuum filtration must be rated for vacuum use (at least 25" Hg, 0.85 Bar, 12.28 psi) and free from scratches, chip and cracks.

Does filtration have to be done in a sterile environment?

Sterilization by filtration is achieved when the filtrate passes through the sterilizing (usually 0.2 µm) membrane into a sterile collection vessel. Until this point, the solution is not considered sterile and does not specifically need to be handled as such, unless the work environment requires this to prevent cross-contamination. The actual collected filtrate should be handled aseptically - removal of the receiver (collection vessel), sealing with the closure and subsequent access to this container should be done with care to maintain sterility.

Can I bubble point test my Nalgene Rapid-Flow filters?

Yes, you can do post-use bubble point testing of all Nalgene Rapid-Flow filter units and bottle top filters. The Bubble Point Test Apparatus (Nalgene cat # DS0405-0050) allows testing of any

Nalgene Rapid-Flow filter without removing the membrane from the filter unit. You must supply a regulated air/gas pressure source and calibrated gauge

What is the hold-up volume for each size Nalgene syringe filters?

Hold-up volumes (after an air purge) are:

4mm	10 µl
13mm	30 µl
25mm	250 µl
50mm	1000 µl

What is the housing material in Nalgene syringe filters?

4mm:	Polypropylene
13 & 25mm:	Modified acrylic for PES, SFCA, CA, prefilter; Polypropylene for Nylon and PTFE
50mm:	Polypropylene

What is the pressure limit (housing burst pressure) of the Nalgene syringe filters?

4mm	75 psig/5.1 bar
13mm	100 psig/7.1 bar
25mm	75 psig/5.1 bar for acrylic housing
25mm	90 psig/6.2 bar for polypropylene housing
50mm	60 psig/4.1 bar

Note: The membranes cannot withstand this pressure and still function as intended. Care must be exercised using small syringes as it is possible to generate pressures in excess of the burst pressure of the syringe filter housings and above the bubble point of the membrane. Use of a membrane above its bubble point pressure can result in a loss of retention/sterility (in the case of a 0.2 µm pore size membrane).

Can you freeze Nalgene Rapid-Flow Filter receiver bottles?

Yes, the polystyrene filter receivers can be frozen; however they are brittle and can crack if dropped. Also be sure to leave at least 20% space for freezing expansion of aqueous fluids. Extreme care must be used when freezing L-glutamine as the solution greatly expands during freezing. It is recommended that L-glutamine solutions be frozen with the bottle at a 45° angle and filled no more than 40% full to minimize the risk of cracking.