

User Guide

Mini-Extruder Assembly Instructions



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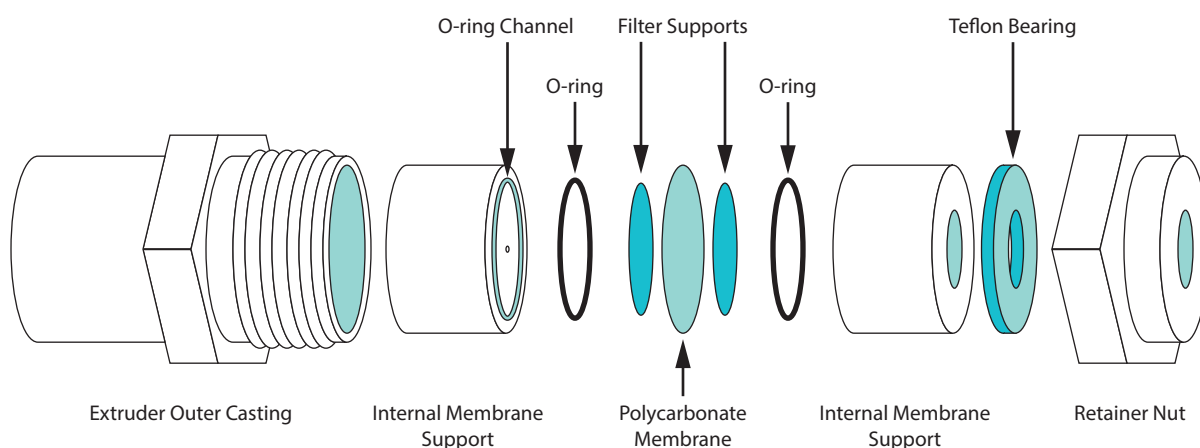
Mini-Extruder Assembly Instructions

Before assembling the Mini-Extruder for the first time, thoroughly clean all parts (except for the polycarbonate membranes and the filter supports) with a mild detergent solution, followed by rinsing with hot tap water and deionized or distilled water. Allow all parts to dry before assembling the apparatus.

The original Mini-Extruder design contains flat washers and a bearing washer that are not stainless steel and will rust if not thoroughly dried. Rinse with methanol or acetone, and dry immediately. The all-Teflon Mini-Extruder design replaces these components with a Teflon bearing. The Teflon bearing is available as a replacement; part number 610019, Teflon washer for extruder.

How To Assemble the Mini Extruder

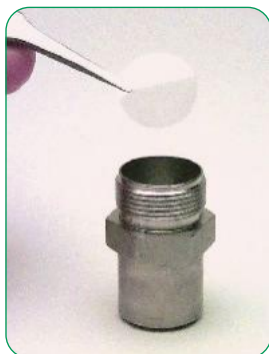
Refer to the diagram to identify the parts in these instructions.



1. Place the two internal membrane supports on a flat surface with the O-rings facing up.
2. Pre-wet two filter supports with DI water or buffer and place them over the orifice. The filter supports should adhere to the Teflon orifice inside the O-ring inner diameter.



3. Insert the internal membrane support, with the filter support, into the extruder outer casing with the O-ring facing up.
4. Place one polycarbonate membrane in the extruder outer casing over the filter support and O-ring. The polycarbonate membrane is the thin, shiny disk—**do not** install one of the blue paper disks that separate the polycarbonate membranes.



IMPORTANT NOTE: Polycarbonate membranes and filter supports are intended to be used for a single liposome preparation and should not be reused.

5. Pre-wet a second pair of filter supports with DI water, or buffer, and place them over the orifice of the remaining internal membrane support.
6. Carefully place the second internal membrane support into the casing (O-ring facing down), being careful not to twist the membrane support when it comes into contact with the membrane.



7. If you have the original Mini-Extruder design, place the flat washers on either side of the bearing washer and insert them into the retainer nut. For the all-Teflon Mini-Extruder, place the Teflon bearing into the retainer nut.



8. Place the retainer nut on the threaded end of the extruder outer casing and tighten. Tighten the retainer nut by hand until it is just finger tight; do not use a wrench.



IMPORTANT NOTE: Autoclaving the Teflon inserts is not recommended as slight distortion may occur (~ 0.002 mm). The use of ethylene oxide or gamma irradiation is suggested as an alternative. The syringes should be sterilized with ethanol.

Warranty

Avanti Research™ guarantees the apparatus free of defects of workmanship for a period of thirty (30) days. If you experience any problems due to workmanship during this time, please contact us to arrange an immediate replacement or exchange of the defective component.

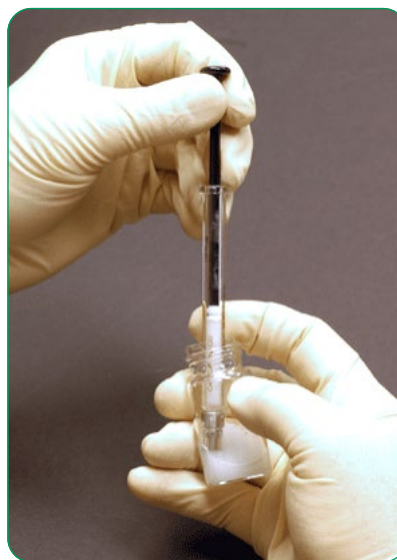
Mini-Extruder Extrusion Technique

NOTE: Extrusion of multilamellar liposomal suspensions using membranes with a pore size $>0.2\ \mu\text{m}$ does not produce unilamellar liposomes. Liposomes produced with larger pore membranes will yield a polydisperse suspension of multilamellar liposomes. Unilamellar liposomal suspensions with low polydispersity can only be prepared with membranes having a pore size of $\leq 0.2\ \mu\text{m}$.

IMPORTANT: Polycarbonate membranes and filter supports are intended to be used for a single liposome preparation and should not be reused.

1. Prepare a dry lipid mixture by lyophilization or evaporation.
2. Place the extruder stand/heating block on a hot plate. Insert a thermometer into the well provided in the heating block. Switch the hot plate on and allow it to reach the desired temperature. Wait approximately 15 minutes for the heating block heating block to reach the desired temperature.
3. Hydrate the lipid mixture using a suitable buffer for at least 30 minutes. The lipid suspension should be kept above the phase transition temperature of the lipid during hydration and extrusion. To increase the efficiency of entrapment of water-soluble compounds, one may subject the hydrated lipid suspension to 3–5 freeze/thaw cycles by alternately placing the sample vial in a dry ice bath and a warm water bath.

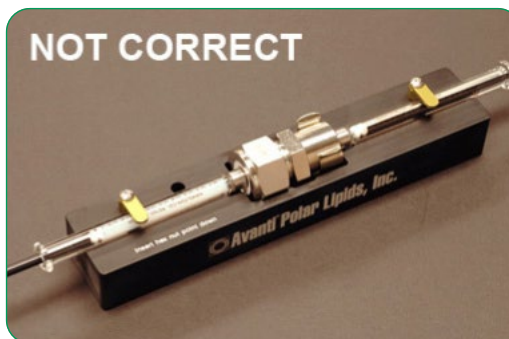
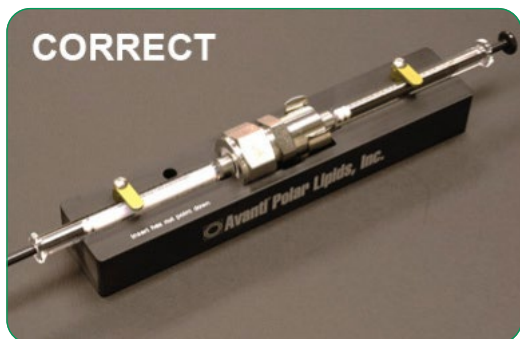
4. Once the sample is fully hydrated, load it into one of the gas-tight syringes and carefully place into one end of the mini-extruder.



NOTE: To reduce the dead volume, pre-wet the extruder parts by passing a syringe full of buffer through the extruder and then discarding the buffer. For new syringes with tight-fitting parts, pre-wet the syringe barrel and plunger with buffer prior to inserting the plunger into the barrel.

5. Place the empty gas-tight syringe into the other end of the Mini-Extruder. Make sure the empty syringe plunger is set to zero; the syringe will fill automatically as the lipid is extruded through the membrane.
6. Check the temperature of the heating block BEFORE placing the assembled extruder apparatus into the heating block. The temperature must be below $80\ ^\circ\text{C}$, or the syringes will be damaged.

7. Insert the fully assembled extruder apparatus into the extruder stand. Insert the hex nut so that the apex of the hex nut points down.



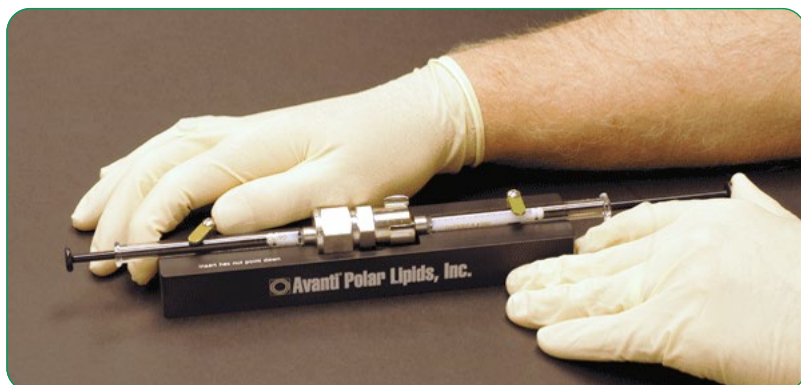
Use the swing-arm clips to hold the syringes in good thermal contact with the heating block.

The extruder apparatus must be fully assembled before inserting it into the heating block; otherwise, it will be damaged. The syringes should fit tightly into the extruder.



8. Allow the temperature of the lipid suspension to equilibrate with the temperature of the heating block (approximately five to ten minutes).

9. Gently push the plunger of the filled syringe until the lipid solution is completely transferred to the alternate syringe.



10. Gently push the plunger of the alternate syringe to transfer the solution back to the original syringe.
11. Repeat the previous two steps. A minimum of four times (for a total of ten passes through the membrane). In general, the more passes through the membrane, the more homogenous the lipid solution becomes.



12. The final extrusion should fill the alternate syringe. This is to reduce the chances of contamination with larger particles or foreign material.
13. After the final extrusion, remove the Mini-Extruder from the heating block.
14. Remove the filled syringe from the extruder and inject the lipid solution into a clean sample vial.
IMPORTANT: When removing syringes, pull the syringe straight out of the extruder; removing at an angle could result in cracking the syringe.
15. Store the vesicle preparation above the transition temperature of the lipid during the experiment. When not in use, store the vesicle solution at 4 °C. Do not freeze. Vesicle solutions are not stable in aqueous media for more than three to four days when stored at 4 °C. Storage of vesicle solutions at higher temperatures and pH 8 may reduce the lifetime of the vesicle suspension.
16. Clean the apparatus thoroughly before using a new lipid preparation.

Care and Cleaning Instructions for Syringes

1. Immediately after use, carefully disassemble both syringes.
2. Depending upon your application, rinse the syringes with either isopropyl alcohol, DI water, or a weak detergent solution. If a detergent solution is used, rinse the syringe immediately with copious quantities of DI water.
3. Rinse the syringes with DI water after cleaning.
4. Dry the syringes.
5. If it is necessary to use a sonic cleaner, only clean the needle with the sonic cleaner. Immediately after cleaning in the sonic cleaner, rinse with DI water and dry.
6. The plunger assembly may need to be replaced if the syringes are used at elevated temperatures for extended periods of time. Replacement parts are available [here](#).

IMPORTANT NOTE: Do not allow the syringes to come into contact with any solvents other than DI water or alcohol. Some organic solvents will interact with the glue holding the threaded insert onto the tip of the syringe, possibly weakening the bond between the barrel and the insert.

Polycarbonate membranes and filter supports are intended to be used for a single liposome preparation and should not be reused.



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Technical Assistance

For more information, please visit **SigmaAldrich.com** for up-to-date worldwide contact information

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