

User manual SETIS™ bioreactor

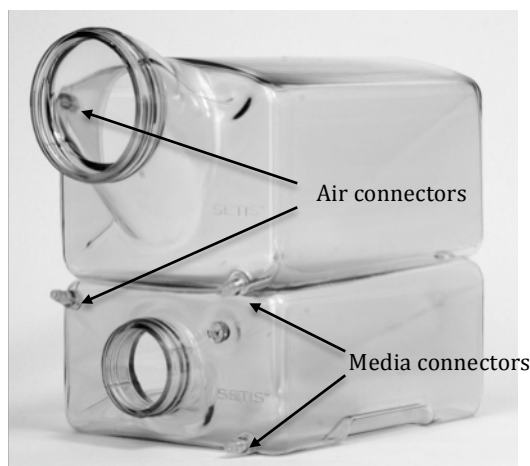
We hope giving enough and clear information in this manual, guiding you through the first trials with SETIS™ bioreactors, without any difficulty. If you require more information do not hesitate to contact us.

Manual last update: May – 2015

Enjoy SETIS™ !!!!

Parts of SETIS™ bioreactor:

- culture vessel (1x) – SE-CV6
- media vessel (1x) – SE-MV4
- screw cap for culture vessel (1x) – SE-C80
- screw cap for media vessel (1x) – SE-C50
- silicone gasket for culture vessel's cap (1x) – SE-SG80
- silicone gasket for media vessel's cap (1x) – SE-SG50
- air filters (2x) – SE-F50
- silicone tube 6/9mm (1x, to be further sectioned in three pieces, see below) – SE-ST6



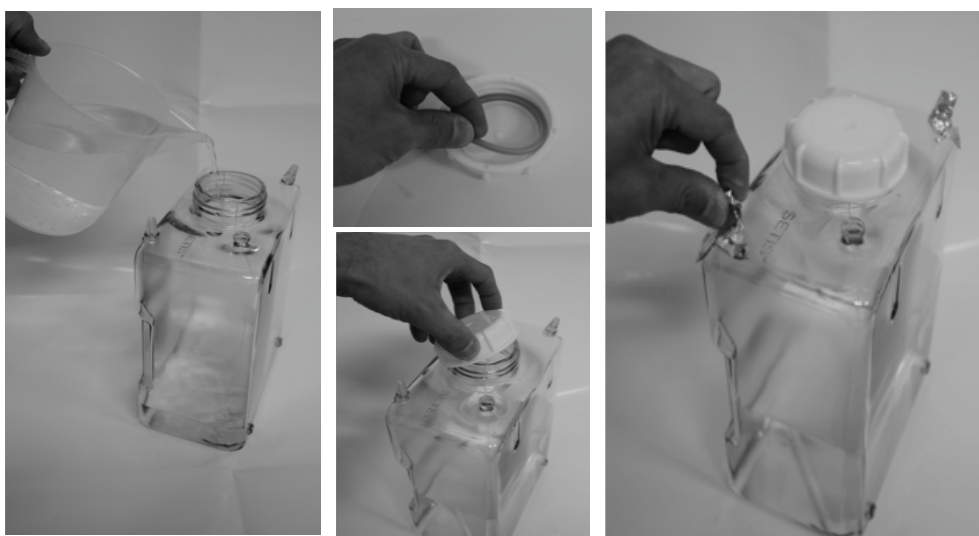
Sterilization

It is important to sterilize all components separated as described in this manual. **DO NOT** sterilizes the bioreactor once assembled, and with air filters connected.

Prepare growth media (liquid) and place it in the media vessel, while standing vertical on its backside. A maximum of 3 liters of growth media is recommended. Bigger volumes could result in growth media entering into the air filter from the vessel, and consequently damaging it. Place the silicone gasket in the corresponding screw cap and close tight the vessel. It is recommended to use the SETIS™ cap's holder (see picture), to guaranty a proper tightness of the vessels. Otherwise, ware gloves to assure a good grip on the caps while screwing.



Cover both connectors (air connector and media connector) with aluminium foil. Sterilize media vessel containing the growth media in the autoclave, preferable 121°C @ 1,2 Atm for 20-25 min. Place the vessels always vertically. Once removed from the autoclave and still hot, screw few millimeters again the cap to guaranty the tightness. **DO NOT** do it, if you have used the SETIS™ cap's holder to tight the caps prior sterilization.



Culture vessel is sterilized empty in the autoclave. Place the silicone gasket in the corresponding screw cap and close tight the vessel. It is recommended to use the SETIS™ cap's holder to guaranty a proper tightness of the vessels. Otherwise, wear gloves to assure a good grip on the caps while screwing.

Cover both connectors (air connector and media connector) with aluminium foil. Sterilize culture vessel in the autoclave, preferable with autoclave program for materials, at 121-125°C @ 1,2-1,4 Atm for 15-20 min.

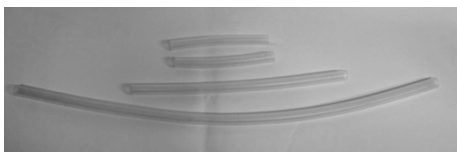


Silicone tube (6/9mm), provided with the bioreactor, is cut in three pieces. Cut two pieces of 8 cm length (used to connect air filters), and one piece of 18 cm (media transfer silicone tube).

The first two pieces are then connected to the air filters provided.

IMPORTANT!!! Connection is made at the **opposite** side of the “IN” side of the filter. Please, take a look at the “IN” sign engraved on the filter’s body. Air filters could remain connected to the silicone tube until the air filters are discarded.

Tubes could then be removed, washed and reused with new air filters.



Air filters are packaged and sterilized inside any autoclave resistant container. They could also be sterilized in plastic bags. **IMPORTANT!!!** For any of the previous options, ventilation should be guaranteed between the inside of the container/bag and the sterilization chamber. If ventilation is not allowed, sterilization might not be efficient or filters could get damaged.

Media transfer silicone tubes are packaged in aluminum foil and sterilized in the autoclave.

Assembly at the Laminar Flow cabinet

Sterile culture vessel is opened in the laminar flow, always facing the airflow. Plant material is then introduced as a bulk.

Close tight the vessel by screwing the cap, avoiding air and/or growth media leakage. It is recommended to use the SETIS™ cap's holder, to guaranty a proper tightness of the vessels. Otherwise, wear gloves to assure a good grip on the caps while screwing.

In case of sub-culturing plant material from SETIS™ to SETIS™, it is then recommended to use plastic stretch foil to cover the cap/neck join of the closed vessel. This will reduce the accumulation of particles while bioreactor is standing on the shelves, hence possible contaminations.

Once plant material is in the culture vessel, place the culture vessel vertically on its backside. Bring the media vessel besides it (also vertically placed), and face the top side of the media vessel with the bottom side of the culture vessel, as indicated in the pictures.

Remove aluminium foil from the air connectors of both containers and place the air filters via the silicone tube. **IMPORTANT!!!** Avoid touching the connectors and edge of the silicone tube with your hands. Once air filters are connected, remove the aluminium foil from the media connectors and place the media transfer silicone tube. Then, is the bioreactor already assembled!!



Change growth media

SETIS™ bioreactors allows an easy, fast and secure way of changing the growth media to plants in growth phase. Follow these instructions to do so.

Fresh growth media is prepared and sterilized in a second media vessel, using the same procedure as explained above.

Bring the SETIS™ bioreactor to the laminar flow and place it vertically. Turn the culture vessel very slowly towards its vertical position, to avoid plant material displacement.

Clean the connectors from old media vessel with disinfection solution.

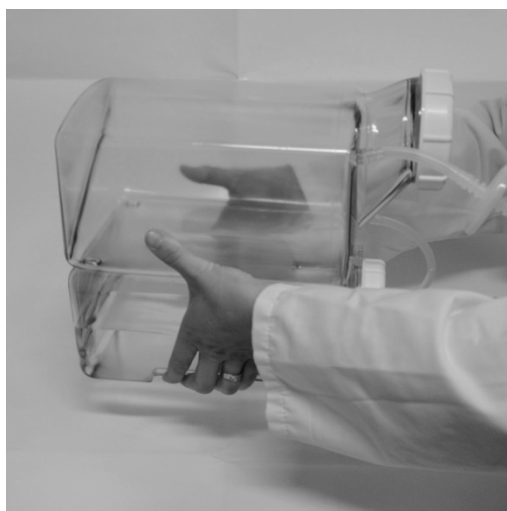
Disconnect the media transfer silicone tube at the side of the media connector of the media vessel. Discard then this media vessel with old growth media. Bring the new media vessel close to the culture vessel and remove the aluminium foil from its media connector. Then, reconnect the media transfer silicone tube to the media connector of the new media vessel. Remove aluminium foil from the air connector of the new media vessel and place a new air filter via the silicone tube.

IMPORTANT!!! Avoid touching the connectors and edge of the silicone tube with your hands. If desired, air filter from the old media vessel could be transferred and reused in the new media vessel.

At the shelf

The topside of the media vessel is complementary to the bottom side of the culture vessel. Via these pins, the culture vessel is stacked and sliding is avoided.

Place SETIS™ bioreactors vertically while transporting with a trolley, otherwise growth media could enter through the air connector of the media vessel. Use the handlers integrated at the bottom of both sides of the media vessel to carry the bioreactor with your hands. Use your thumbs to hold the culture vessel as shown in the picture.



Place the bioreactors on a perfectly horizontal and hard shelf surface. The bioreactor has the required inclination to allow self-drainage followed the

immersion phase. This inclination can be altered by irregular and/or wavy shelf-surfaces.

Place the bioreactor with the front side (where all connectors and caps are present), facing the outside of the shelf (corridor). Connect both filters with their respective compressed air line. Now is the bioreactor ready to operate!!!



SETIS™ bioreactors are designed to be placed side by side, and back to back, without losing any shelf space, with the advantage of having all connectors and caps at the outside of the shelf.



Installation

SETIS™ racks are made to fit 16 SETIS™ bioreactors per shelf (two rows of 8). A standard rack setup is composed of 4 shelves, with a total of 64 SETIS™ bioreactors. All required pneumatic connections are supplied with the SETIS™ rack.

In case users adapt their lab racks for SETIS™, we recommend connecting a maximum of 8 bioreactors per row and a maximum of 4 rows in parallel per Control Point. In total, a maximum of 32 SETIS™ bioreactors per Control Point (Based on SETIS™ Control unit). **IMPORTANT!!!** Design and calculate your compressed air supply based on the desired rack setup, therefore avoiding bioreactor's malfunction.

SETIS™ bioreactor has been designed with a minimum height difference between the media connectors of both vessels. Hence, the air pressure required to perform an immersion is very low. Working air pressure is between 0,1 – 0,3 BAR. Preferably 0,1 BAR.

Higher pressures might result in vessel cracking, hence leakages.

General remarks

Working with liquid systems requires a careful handling under sterile conditions, as contaminations may easily outgrow. During the first trials with SETIS™ bioreactors, perform slowly all activities under sterile conditions, in order to reduce mistakes and unnecessary contaminations. The 'Practice' is the best method to become an expert handling SETIS™ bioreactors.

Silicone gaskets are designed to enter tight into the inner side of each cap. This is to avoid that the silicone gasket falls from the cap once the vessel is opened under sterile conditions. To place the silicone gasket correctly, just place it into the cap and screw it to its corresponding vessel. The edge of the vessel's neck will push the silicone gasket uniformly to its place. **IMPORTANT!!!** Avoid any loop in the silicone gasket prior screwing the caps. This will result in vessel leakages.

Wash the vessels thoroughly using neutral detergent. Aggressive detergents can induce cracks in the vessels body, which will increase after several sterilization cycles. Rinse them afterwards with clean water, preferably demineralized water. Avoid vessels to get in contact with solvents and oils. Perform a periodic cleaning of your autoclave.

Liquid media is ideal for bacterial growth. Endogenous bacteria, which have been never detected in agar cultures, could excessively grow in liquid cultures such as in SETIS™ bioreactors. Control and diagnostic very good your starting materials against endogenous bacteria before you inoculate a SETIS™ bioreactor.

SETIS™ bioreactor is designed to place the culture vessel on top of the media vessel. They will be solely connected via the media transfer silicone tube, through both media connectors. Make sure this connection is properly done, without leakage. Avoid twisting or bending the media transfer silicone tube.