User Manual SETIS™ bioreactor

We hope to provide you with sufficient and clear information in this manual to guide you through the first trials with SETIS™. It is highly recommended to review our FAQs, additional manuals, technical information, and instruction videos on our website.
Please, visit www.setis-systems.be, where you'll find vast information about our products and procedures.

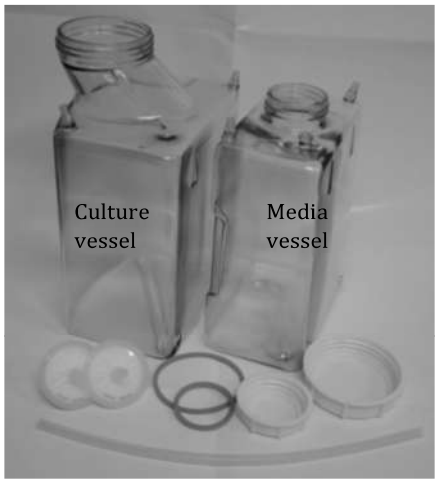
If you require any further information, please do not hesitate to contact us.
Manual last update: February - 2020

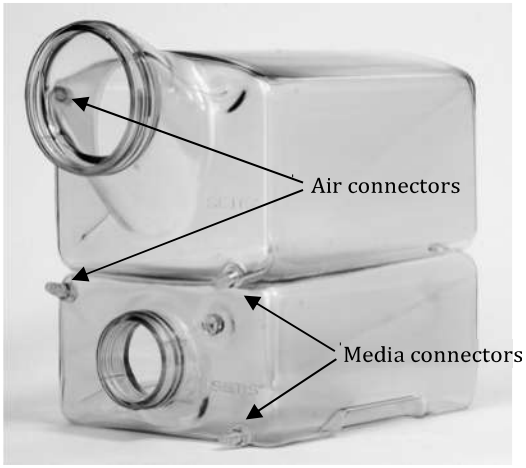
Enjoy SETIS™ !!!!

Each SETIS™ bioreactor is delivered with the following parts:

- 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 6/9Ømm (1x, to be further sectioned in three pieces, see below) – SE-ST6

More detailed information about all parts can be found at: https://setis-systems.be/products/setis-bioreactor





Washing

SETIS™ bioreactors are designed to be reused many times. If the vessels are handled correctly, the bioreactor can be used for many years.
Washing is one of the essentials steps in this process. Before using the bioreactor, even before first use, all parts need to be washed.

Vessels
SETIS™ vessels are made of polycarbonate, a very hard plastic that needs special care during washing to ensure a long lifespan.
Wash the vessels thoroughly with neutral detergent (pH 7). Never use alkaline or other aggressive detergents. They can damage the plastic (polycarbonate) and induce cracks in the vessels' body, which can deteriorate after several sterilization cycles. Rinse the vessels afterwards with sufficient tap water to remove all detergent traces. Do not use soft water produced by a salt-softener machine. Excess of sodium in water generated by these

VERVIT bv
Karnemelkstraat 2-4, 9060 Zelzate, Belgium
T: +32486918701 info@vervit.be www.vervit.be

machines can affect the plastic. At last, you can rinse the vessels with demineralized water, if possible (not strictly necessary).
Prevent the vessels from getting in contact with solvents and oils. Periodically clean your autoclave.
Spots and stains on the vessels, generated by phenols, charcoal or other pigments, can be cleaned out using bleach. If this is the case, opt for a solution with low bleach concentration and adjust pH to 7. Pour the solution in the vessels and place the vessels on a shaker until the spots disappear. Then wash the vessels.
Use soft sponges and brushes to wash the vessels. Never use scrape sponges as they will damage the plastic surfaces. As long as they use appropriate detergent and rinse water, washing machines are also suitable.
Once the vessels are washed, let them dry in a clean room. Never place vessels in a drying oven. It will damage the plastic and glue used for the connectors.

Other parts

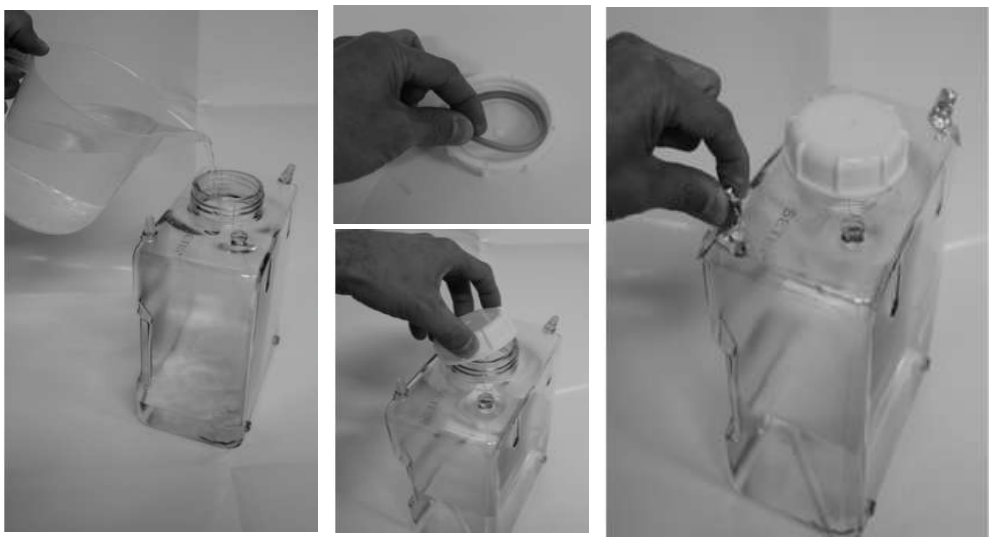
The remaining SETIS™ components are made of more resistant plastic (Polypropylene and Silicone). They can be washed with any detergent, although it is recommended to use the same neutral detergent as for the vessels. (As traces of alkaline detergents in these parts could still affect the vessels, once assembled for sterilization.)
Use soft sponges and brushes to wash these parts. Never use scrape sponges, they will damage the plastic surfaces. Washing machines are also suitable, as long as they use appropriate detergent and rinse water.
Silicone tubes, used for transfer media, can get quite dirty after several use cycles. If so, they may be immersed in a bleach solution as well, preferably on a shaker, until they're clean. Afterwards, wash and rinse, as described.
As soon as all parts are clean and dry, store them in a clean or closed environment until further use. Store the vessels, during and after preparation for sterilization, in a clean and closed environment as well to prevent dust from entering the vessels.

Preparation for sterilization

Vessels

Prepare (liquid) growth medium and pour it into the media vessel, while the vessel stands vertically on its backside. A maximum of 3 litres of growth media is recommended. Bigger volumes could result in growth media entering from the vessel into the air filter, and consequently damaging the filter. Place the silicone gasket in the corresponding screw cap and firmly tighten the vessel. It is recommended to use the SETIS™ caps holder (see picture below), to guarantee a proper tightness of the vessels. Otherwise, wear gloves to assure a good grip on the caps while screwing. Thereafter, cover both connectors (air connector and media connector) with aluminium foil.

VERVIT bv
Karnemelkstraat 2-4, 9060 Zelzate, Belgium
T: +32486918701 info@vervit.be www.vervit.be



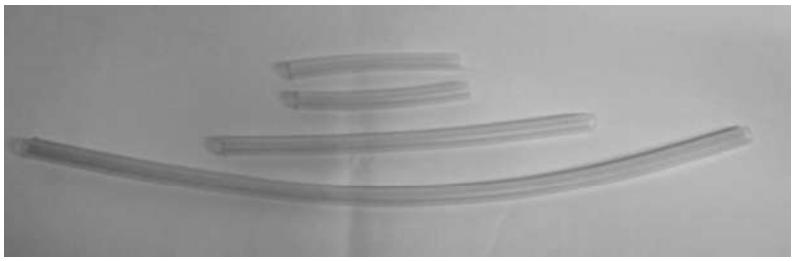
The Culture vessel is sterilized empty in the autoclave. Place the silicone gasket in the corresponding screw cap and firmly tighten the vessel. It is recommended to use the SETIS™ caps holder to guarantee a proper tightness of the vessels. Hold the vessel with your hand and keep the lateral side of the vessel on the table while closing the caps. If the caps holder is not available, wear gloves to assure a good grip on the caps while screwing. Cover both connectors (air connector and media connector) with aluminium foil.

Silicone gaskets are designed to fit tight into the inner side of each cap, to prevent the silicone gasket getting loose from the cap, once the vessel is opened under sterile conditions. Place the silicone gasket into the cap and screw it onto its corresponding vessel. The edge of the vessel's neck will push the silicone gasket uniformly into its place.
IMPORTANT!!! Avoid any loop in the silicone gasket prior to screwing the caps. This will result in vessel leakages.

Silicone tube and Air filters

Silicone tube (6/9Ømm), 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).
Then, the first two pieces are connected to the air filters. IMPORTANT: Connection is made at the opposite side of the "IN" side of the filter. Please, take notice of the "IN" sign engraved on the filter's body. Air filters can remain connected to the silicone tube until the air filters are discarded. Tubes can then be removed, washed and reused with new air filters.

VERVIT bv
Karnemelkstraat 2-4, 9060 Zelzate, Belgium
T: +32486918701 info@vervit.be www.vervit.be



Air filters are packaged and sterilized inside any autoclavable resistant container. Preferably use hard-plastic containers such as PP or PC. Make sure that for any container used, air exchange is guaranteed during sterilization. Silicone tubes must not be bent inside the container. You can also wrap the filters / silicone tubes in aluminium foil or paper.

Do not use plastic bags, as they could deform or block air exchange with the autoclave chamber, compromising sterilization and filter membrane integrity.
IMPORTANT: Air exchange between the inside of the container and the sterilization chamber should be guaranteed.

Like the air filters, media transfer silicone tubes are wrapped in aluminium foil or packed in any hard-plastic container. Tubes must not be bent inside the container.

Sterilization

All SETIS™ parts are autoclavable. It is important to sterilize the components separately as described in this manual.
Sterilize each vessel with its corresponding screw cap screwed on and the connectors covered with aluminium foil. DO NOT sterilize the bioreactor once assembled or with air filters connected.
Place the vessels in vertical position inside the autoclave. Make sure they're positioned in a stable way to prevent them from falling due to vibrations inside the chamber. Air filters and tubes can be distributed in any space in the autoclave.
Label materials with autoclave tape prior sterilization to later identify sterile materials.

Media vessel sterilization (including media): 121°C @ 1,2 Atm. for 20-25 min.
Culture vessel (empty): 121-125°C @ 1,2-1,4 Atm. for 15-20 min.
Silicone tubes and air filters: 121-125°C @ 1,2-1,4 Atm. for 15-20 min.

Do not use autoclave programs that include vacuum cycles, indicated in some autoclaves as "material program". This can damage the vessels (deformations, cracks in plastic, loose connectors) and the membrane of the air filters. For autoclaves with a separate steam tank, heating and cooling phases should be slow, otherwise vessels could also be damaged. Specifically, the cooling phase should take at least 20-25min from 121°C to 90°C.

Once autoclaved, leave materials to cool down in a clean room until further use.
After removing the vessels from the autoclave: Do not tighten the screw caps of the vessels again, while they're still hot. It will deform the caps and break them. Caps should only be tightened when vessels are at room temperature.

Assembly at the Laminar Flow cabinet

Open the sterile culture vessel, facing the airflow, in the laminar flow. Introduce plant material in bulk into the culture vessel. Firmly tighten the vessel by screwing the cap, to prevent air and/or growth media leakage. It's recommended to use the SETIS™ caps holder to guarantee a proper tightness of the vessels. If the caps holder is not available, wear gloves to assure a good grip on the caps while screwing.

VERVIT bv
Karnemelkstraat 2-4, 9060 Zelzate, Belgium
T: +32486918701 info@vervit.be www.vervit.be

Working with liquid systems requires a careful handling under sterile conditions, as contaminations may easily spread. Execute all activities slowly under sterile conditions during the first trials with SETIS™, in order to reduce mistakes and undesired contaminations. Through practice, you will become an expert in handling SETIS™ bioreactors.

Once the plant material is in the culture vessel, place the culture vessel vertically on its backside. Bring the media vessel (also vertically positioned) beside it and position the top side of the media vessel towards the bottom side of the culture vessel, as indicated in the picture.

Remove aluminium foil from the air connectors of both vessels and attach 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 attached, remove the aluminium foil from the media connectors and attach the media transfer silicone tube. After this step, the bioreactor is successfully assembled.



To attach the silicone tubes correctly to the connectors, push the tube over the connector while slightly screwing it until you reach the second grip of the connector. Do not push the tube any further, as it would be difficult to remove later. The opposite action is required to remove the tube from the connector: gently pull the tube while screwing it out.
SETIS™ culture vessels are designed to be placed on top of the SETIS™ media vessels, solely connected via the media transfer silicone tube, by both media connectors. Make sure this connection is properly executed, without any leakage. Avoid twisting or bending the media transfer silicone tube.

Changing growth media

VERVIT bv
Karnemelkstraat 2-4, 9060 Zelzate, Belgium
T: +32486918701 info@vervit.be www.vervit.be

One of the advantages of SETIS™ bioreactors is an easy, fast and secure change of growth media to plants during their growth phase.
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 the old media vessel with disinfection solution. Disconnect the media transfer silicone tube from the media connector of the media vessel. Remove the 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, the air filter from the old media vessel can be transferred to 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 them 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 below.

Place the bioreactors on a perfectly horizontal and hard shelf surface. The bioreactor has the required inclination to allow self-drainage following the immersion phase. This inclination can be altered by irregular and/or wavy shelf-surfaces, compromising a correct media drain.
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 the bioreactor is ready to operate!!!

VERVIT bv
Karnemelkstraat 2-4, 9060 Zelzate, Belgium
T: +32486918701 info@vervit.be www.vervit.be

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 designed to accommodate 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 (SE-R64 & SE-R32). In case users adapt their own 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 to avoid bioreactor's malfunction.
SETIS™ bioreactors have been designed with a minimum height difference between the media connectors of both vessels (only 10cm). Hence, the air pressure required to perform an immersion, displacement of growth media from media vessel to culture vessel, is very low. Working air pressure should be between 0,1 – 0,2 BAR. Preferably 0,1 BAR. Higher pressures might result in bioreactor malfunctioning, vessel cracking and leakages.

VERVIT bv
Karnemelkstraat 2-4, 9060 Zelzate, Belgium
T: +32486918701 info@vervit.be www.vervit.be