sartorius stedim biotech

Manual

Sartoclear Dynamics®

85037-548-07

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Contents

Introduction

This manual provides a detailed description of all installation and handling aspects of the Sartoclear Dynamics® product range. Additionally this document contains background information on dynamic body feed filtration.

Liability

The products described in this document are intended to be used for the clarification of mammalian cell cultures. Sartorius Stedim Biotech cannot assume liability if Sartoclear® products are subjected to improper use. The use of this product for other applications or in another manner as described in this manual may result in personal injury or malfunction of the products. Please follow the user manual for your own safety and to obtain optimal filtration results.

Sartoclear Dynamics® DE Bags contain Celpure® C300 Diatomaceous Earth from the company Advanced Minerals. Celpure® C300 is a calcined diatomaceous earth and contains crystalline silica of which a small fraction (less than 1%) can be classified as respirable crystalline silica (RCS), which is the fraction that can enter the lung alveoli. Prolonged and | or massive inhalation of respirable crystalline silica dust may cause lung fibrosis, commonly referred to as silicosis and associated forms of lung cancer. Occupational exposure to RCS dust should be monitored and controlled.

Hazard classification according to the global GHS System is based on its Respirable Crystalline Silica content. C300 contains less than 1% of RCS and thus does not require classification as hazardous substance.

Moreover, Sartoclear Dynamics® provides a system for dust free transfer of Celpure® C300 to a liquid. It has been qualified as technical means to fulfill the strictest current occupational exposure limits for RCS when used correctly. Please read this manual carefully before using this product. It is recommended to train all personal who may have contact with the product or its waste.

Please refer to the validation guide for detailed information on the qualification procedure of the powder transfer system.

In the interest of product development we reserve the right to make changes.

Reference List

A

Sartoclear Dynamics® DE Bags

DE Content	Quantity	Order Number
0.5 Kg	4	29Z-B00005
1.5 Kg	2	29Z-B00015
5 Kg	2	29Z-B00050
10 Kg	1	29Z-B00100

B

Sartoclear Dynamics® KIT

Order Number
29Z-K00001

Sartoclear Dynamics® KIT contents:

Filterline

B1

Powder Transfer Adapter

B2

Zipper

B3

Clamps for powder transfer

B4

C

Flexel® 3D Magmix Bags

Volume	Quantity	Order Number
100 L	2	FMB122965
200 L	2	FMB123485
400 L	2	FMB123486
650 L	1	FMB123487
1,000 L	1	FMB122973

Additional Equipment for DE Bags

D

D1

Description	Order Number
Fork lift **(D)**	2ZG---0003
Adapter **(D1)**	2ZG---0004

Sartoclear Dynamics® Filter

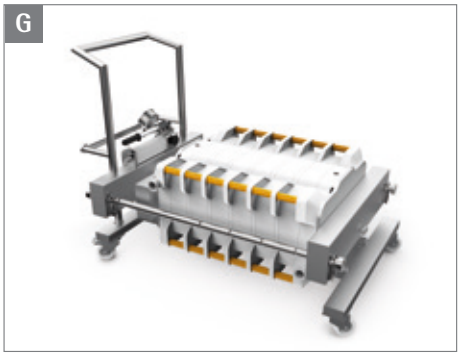
E

Description	Order Number
Sartoclear Dynamics® Filter	29Z-H00001

F

Description	Order Number
Sartoclear® Manifold Set	29Z-S00001

G



Sartoclear® Pilot Filter Holder

Description	Order Number
Sartoclear® Pilot Filter Holder for 1 – 6 × 0.21 m² Sartoclear Dynamics filters	2ZGL--0005
Drip Pan for Sartoclear® Pilot Filter Holder	2ZGL--0008

Directions for Use

1. Material

Sartoclear Dynamics® is a group of products which together form a complete unit operation. A complete list for reference can be found above.

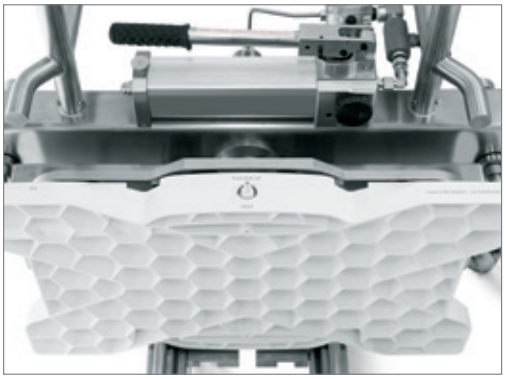
2. Installation descriptions

1. Installation of the Sartoclear Dynamics® Filters **(E)** and the Sartoclear® Manifold Set in the Filter holder **(G)**
2. Installation of the Powder Transfer Adapter **(B2)** (part of the Sartoclear Dynamics® Kit **(B)**)

2.1 Installation of the Sartoclear Dynamics® Filters and the Sartoclear® Manifold Set in the holder

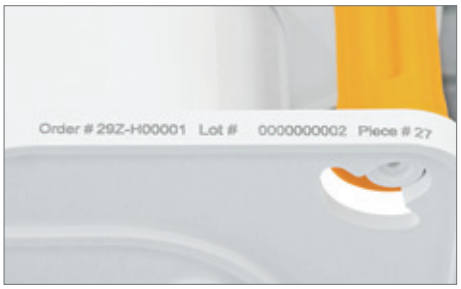
▶ Connect ¼ inch ID ∅ mm tubing with a clamp to the drains and the vents of the manifold set **(F)** so that the DRAIN and VENT can be opened and closed during operation.

▶ Place the INLET plate of the manifold set at one end of the holder in such a way that the text "THIS SIDE UP" is visible from above.



▶ Place the required amount of Sartoclear Dynamics® Filters between the INLET and the OUTLET plate. The text "THIS SIDE UP" and the Order and Lot# should be visible from above.

The optimal amount of Sartoclear Dynamics® filters is relative to the total wet cell weight (WCW) and the applied DE concentration (see also: background information on page: 10). As a rule of thumb 1.5 kg of DE per Sartoclear Dynamics® filter can be considered. If you need further assistance please contact your local Sartorius Stedim Biotech representative.



▶ Place the OUTLET plate at the other end of the holder in such a way that the text "THIS SIDE UP" is visible from above.

IMPORTANT

Please see the manual of your filter holder (Sartoclear® Pilot Filter Holder manual: 85037-547-73) for adjustment of the system to the correct number of filters and adjustment of the clamping force.

▶ Clamp the Sartoclear Dynamics® Filters together with the IN- and OUTLET plates with a clamping force of **25 kN**.

▶ Connect the inlet and outlet tube to the inlet plates with 1½" tri-clamp (TC)

▶ After applying a clamping force of 25 kN the silicone seals inside the Sartoclear Dynamics® Filters slowly settle. The result is a decay of the clamping force. It is necessary to allow settlement for at least 10 min. The clamping force must be readjusted to 25-30 kN just before the start of the filtration

2.2 Installation of the Powder Transfer Adapter Kit

IMPORTANT

Install the Powder Transfer Adapter (B2) out of the Sartoclear Dynamics® KIT (B) on top of the Flexel 3D Magmix Bag (C) (in this document called: Magmix Bag) before the Magmix Bag is installed in the Palletank® for Magnetic Mixer.

▶ Remove the 8" white plastic cap from the Magmix Bag by loosening the tri-clamp

▶ Take care that the transparent gasket stays in place

▶ Put the Powder Transfer Adapter **(B2)** on the gasket

▶ Replace the tri-clamp

▶ For installation of the Magmix Bag please follow the instructions for installation of the Magmix Bag.

▶ If you need to calibrate the pH sensor, follow the directions for use of "pH probe calibration and insertion in a Flexel® 3D Bag for LevMixer® | Magnetic Mixer".

3. Process descriptions

1. Transfer of cell culture fluid
2. Pre-conditioning of the cell culture fluid (Optional)
3. Addition of the filter-aid
4. Filtration
5. Recovery optimization
6. Draining of the system
7. Disassembling

3.1 Transfer of the cell culture fluid

▶ Connect the Bioreactor to the Magmix Bag and pump the cell culture fluid into the Magmix Bag with a suitable pump and start gentle mixing.

3.2 Pre-conditioning of the cell culture fluid (Optional)

Sartoclear Dynamics® products can be applied immediately after stopping the cell culture. However, if possible, it is recommended to pre-condition the cell culture fluid by precipitation or flocculation in order to improve the performance (See also Box with background information).

▶ Use the long (2 m) silicone inlet line on top of the Magmix Bag for the addition of acids, bases or flocculants.

Never use the Powder Transfer Adapter to add acids, bases or flocculants. This port can only be used in combination with Sartoclear Dynamics® DE Bags.

Background information
Adding diatomaceous earth to the filtration process has two purposes: Its porous structure keeps the filter cake permeable and it sieves out sub-micron particles to protect subsequent membrane filters.

The amount of Celpure® C300 you mix into your cell culture is relative to your biomass. Based on a large number of tests with many different cell cultures we recommend the following DE|biomass (WCW) ratios.

– pH 7: 40-50%
– pH 5: 25-30%

Depending on the precipitation | flocculation procedure, it may take some time until the reaction is finalized. For this reason it is recommended to start the precipitation | flocculation before addition of the filter-aid.

Example: Low pH precipitation
A fast and effective example of pre-conditioning the cell culture fluid is low pH precipitation. Best results were reported with organic acids such as 2 M acetic acid to pH 5 and 45 minutes of mixing.

3.3 Addition of the filter-aid

▶ Connect the Filterline **(B1)** to the Palletank® for Magmix Bags.

▶ Open the quick connector on the Magmix Bag and connect the Filterline. Take care that the vent filter does not get in contact with the liquid.

IMPORTANT

In order to minimize the shear stress on cells it is recommended to add the filter-aid just before the start of the filtration. After powder addition it takes only 5 – 10 minutes to suspend the DE.

▶ Use the Sartoclear Dynamics® KIT **(B)** to connect the Sartoclear Dynamics® DE Bags **(A)** to Sartorius Magmix Bags **(C)**.

▶ Please confirm that your Forklift **(D)** has the correct adapter **(D1)** as shown on the picture.

IMPORTANT

Sartoclear Dynamics® products are designed to protect the environment for dust. Please always obey the following rules while working with Sartoclear Dynamics® products to avoid dust formation:
– Always handle the products with care
– Never use sharp tools in the proximity of bags
– Always put DE bags (full and emptied) in a safe place to avoid accidents
– Never use strong forces to open or close bags
– Dispose emptied DE-Bags as soon as possible
– Never use damaged bags
– Seal damaged bags immediately
– Use water to avoid dust formation in cases powder bags are damaged

▶ Hang a Sartoclear Dynamics® DE Bag on the Forklift

▶ Bring the DE Bag in the right position above the Powder Transfer Adapter.

▶ Take the Zipper **(B3)** and insert the rails of the Sartoclear Dynamics® DE Bag in the Zipper. Around 1 cm of the rails should come out.
(See also 3d-print on the Zipper for a correct insertion of the rails).

▶ Insert the rails of the Powder Transfer Adapter in the other inlet of the Zipper so that the rails of the Sartoclear Dynamics® DE Bag and the Powder Transfer Adapter seal together.

▶ Hold the two ends of the rails together and pull the Zipper. The Sartoclear Dynamics® DE Bag and the Powder Transfer Adapter are now connected.

▶ Bring four fingers behind the plastic lip on the Sartoclear Dynamics® DE Bag below the text "Open here" on the yellow folding plate. Bring your other hand behind the lip on the Powder Transfer Adapter.

▶ Bend your fingers and pull so that the rail opens. The rail should open easily. The port of the connected Sartoclear Dynamics® DE Bag and the Magmix Bag is now opened.

▶ Push the yellow folding plates inwards so that the plastic film completely covers the rails to prevent that DE will stick to the rails.

IMPORTANT

If powder sticks to the rails, disconnection of emptied bags and connection of new Sartoclear Dynamics® DE Bags becomes more difficult.

▶ Use the four Clamps for Powder Transfer **(B4)** to keep the plastic film in position and to protect the rails during powder transfer. Gently push the clamps in the corners for the best protection.

▶ The yellow silicone lips should go over the rails on the outside of the Powder Transfer Adapter.

IMPORTANT

The Filterline (B1) must be opened before you release the powder.

▶ As soon as the Clamps for powder transfer are positioned correctly the plastic bar can be opened to release the powder.

▶ Gently shake the DE Bag up and down to speed up the transfer of DE

▶ In order to speed up powder uptake you may temporarily increase the stirring speed of the Magmix drive unit. See also table 1 for the recommended mixing speed per suspension volume for suspending the DE in the cell culture fluid.

Table 1: Recommended mixing speed per volume

Suspension volume (cells & DE)	100 L	200 L	400 L	650 L	1,000 L
Recommended mixing speed*	50 rpm	70 rpm	90 rpm	110 rpm	125 rpm

* The recommended mixing speed is sufficient to maintain a homogenous suspension of cells and filter-aid. Increased mixing speed may increase the shear stress on the cells

▶ Remove the 4 clamps and pull on the film of the emptied DE Bag so that the yellow folding plates come up.

▶ DE is a very porous material filled with air. By suspending the DE the entrapped air is released and will fill up the emptied DE Bag. The air can be released via the Filterline on the Magmix Bag.

▶ Take the plastic bar from the hook and roll the bag around the bar. Excess air is pushed back in the Magmix Bag and can be released via the Filterline.

IMPORTANT

Never use force on the Zipper or Powder Transfer Adapter. The Zipper should move easily. If you feel a resistance DE may be stuck in the rails of the adapter. Move it in the reverse direction and try to remove the DE before you disconnect the bags.

IMPORTANT

Sartoclear Dynamics® products are designed to protect the environment for dust. Please always obey the following rules while working with Sartoclear Dynamics® products to avoid dust formation:
– Always handle the products with care
– Never use sharp tools in the proximity of bags
– Always put DE bags (full and emptied) in a safe place to avoid accidents
– Never use strong forces to open or close bags
– Dispose emptied DE-Bags as soon as possible
– Never use damaged bags
– Use water to avoid dust formation in cases powder bags are damaged

▶ Disconnect the emptied DE Bag by pulling the Zipper backwards. The Powder Transfer Adapter and the emptied DE Bag will be closed and disconnected.

WARNING

Check if the rail of the powder transfer is completely closed before you connect a new DE Bag. A maximum of 5 DE Bags per Powder Transfer Adapter can be used.

▶ Start the filtration after the DE is completely suspended (5 – 10 minutes).

Samples:
DE blocks the septum of the clave connector and in case you would like to take samples from the suspension the connector (blue) has to be removed. A standard Luer Lock syringe can be used to take samples.

3.4 Filtration

▶ Make sure that both drains on the Manifold Set are closed and start the filling of the Sartoclear Dynamics® Filters with a constant flow rate of 300 L/m²/h.

▶ Re-adjust the clamping force to 25-30 kN just before filtration.

▶ Close the vents on the IN- and OUTLET plates when the housings are filled (fluid will appear in the tube).

▶ The maximum operating pressure during filtration is 1.5 bar (21.75 psi)

▶ The maximum allowed back pressure on the Sartoclear Dynamics® filters is 0.7 bar (10.15 psi)

▶ The temperature of the fluid must be between 5 – 40° C

For an easier handling of your emptied Magmix Bag after use you can close the vent filter at 50% volume capacity so that the Magmix Bag shrinks during processing.

- ▶ Switch the pump to constant pressure mode if the pressure reached 1.5 bar (21.75 psi)
- ▶ Stop the filtration when the flow rate goes down to 150 L/m²/h

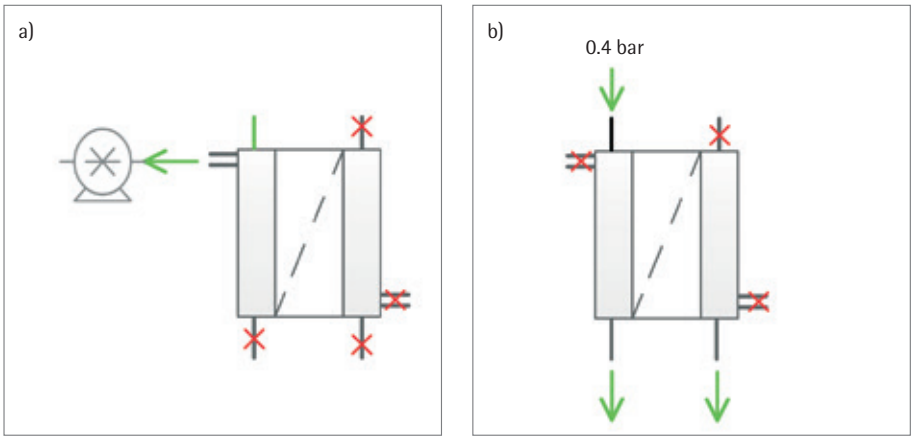
IMPORTANT

In cases the filtrate has to be neutralized for the subsequent unit operation it is recommended to neutralize during filtration with NaOH (1M) (approximately 10 L/m²/h) to obtain best results. The 2.5 m long inlet tubes on the Magmix Bag can be used for this purpose.

3.5 Recovery optimization

- ▶ Connect a container with a suitable buffer for your application to the filtration set up
- ▶ Start flushing the system with constant flow or constant pressure (max 1.5 bar (21.75 psi)).
- ▶ Optimal results are obtained obtained with a buffer volume of around 7.5 L per Sartoclear Dynamics® Filter.

3.6 Draining of the system



- ▶ Sartoclear Dynamics® Filters **must** be drained before the clamping force is released
- ▶ Follow the steps a) and b) as shown in the schematic overview above.

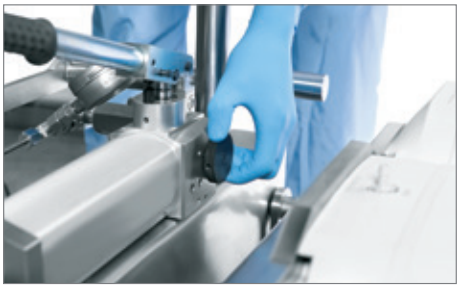
a) Close all openings (DRAIN, VENT and OUTLET) on the OUTLET plate. Open the VENT on the INLET plate and pump in reverse direction to remove the remaining buffer.

b) Close the INLET. Connect both drains of the IN- and OUTLET plate to an appropriate container for collecting the fluid. Open the drains of the IN- and OUTLET plate to DRAIN the filter. Pressurized air (max 0.4 bar or 5.8 psi) on the VENT of the INLET plate can be used to enhance the draining process.

3.7 Disassembling

▶ In order to prevent spilling of fluid on the floor an optional drip pan (2ZGL--0008) can be used.

▶ Release the pressure from the hydraulic clamping system (see the manual of your filter holder)

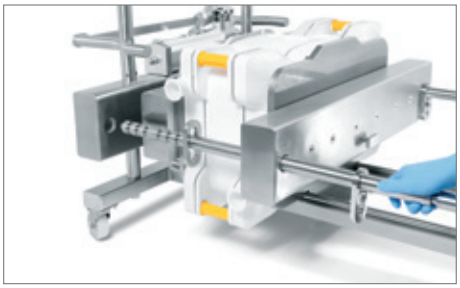


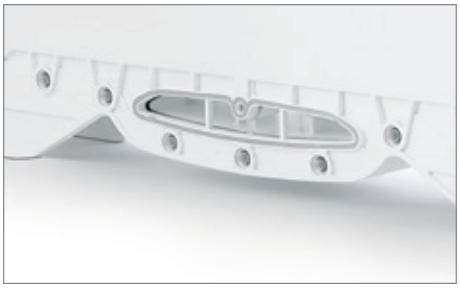
▶ For Sartoclear® Pilot Filter Holder (on the picture) the pressure can be released by turning the black rounded button to the left (see also the Sartoclear® Pilot Filter Holder manual: 85037-547-73).

CAUTION

Single Sartoclear Dynamics® Filters occasionally may reach a total weight of more than 15 Kg. Use the ergonomic design of the Sartoclear® Filter Holder and the Sartoclear Dynamics® Filters for a safe off-loading of heavy filters.

▶ For Sartoclear® Pilot Filter holder the positioning bars have to be removed for an easier off-loading of heavier filtration devices.

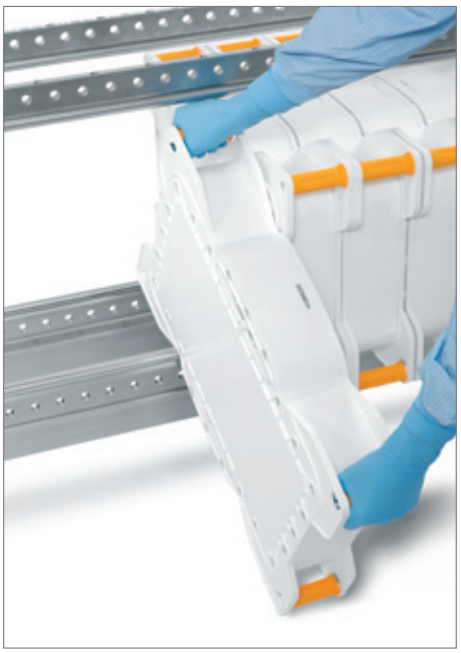




▶ Sartoclear Dynamics® Filters have two grooves to fit on the rails of filter holders. A special rounded edge between the grooves supports an ergonomic off-loading of heavy filters.

▶ Lift and pull the filters in such a way that the rounded edge on the bottom edge of the filter is supported by the rail that is closest to you.

▶ Pull the filter out of the holder until the second groove reaches the first rail.



▶ Push the filter down so that the second grip can be reached for easy handling.

Disposal

All components of the Sartoclear Dynamics® are non-hazardous and can be disposed through normal waste removal methods, in accordance with the local legislation. The disposal method of the Sartoclear Dynamics® Filters may depend on the nature of the residual material originating from the filtered material.

C300 leakage from the DE bags and dust formation must be avoided during disposal. Sartorius recommends to contact unused diatomaceous earth with water to prevent dust formation prior to disposal. Check your local regulations for advised disposal procedures.

Applications Support

Contact your local Sartorius Stedim Biotech representative to obtain general information and technical data. We will be more than happy to assist your filtration trial with one of our application specialists around the globe.

Return of used products

In the case where a return of the materials to Sartorius Stedim Biotech is necessary, please ensure these materials are properly sterilized prior to shipment back to Sartorius Stedim Biotech. This allows our staff to handle them with minimal risk during the inspection of products. The law requires that a return shipment form (available through your local Sartorius Stedim Biotech representative) must be completed prior to shipping of used filter materials.

Thank you for working with Sartorius Stedim Biotech, we appreciate your business.

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