sartorius stedim biotech

High cell density *Escherichia coli* cultivation in the stirred single use bioreactor BIOSTAT® CultiBag STR 50 plus MO

Application Note

#02
#03
#04
#05
#06

turning science into solutions

Thomas Dreher, Ute Husemann,
Gerhard Greller, Jörg Weyand

Sartorius Stedim Biotech, GmbH,
August-Spindler-Str.11,
37079 Goettingen, Germany

Single use products offer many advantages in manufacturing processes, due to this they have become widely accepted for cell cultural applications (Eibl et al., 2009). Nowadays, reports about microbial application are rarely found in single use bioreactors, commonly because the oxygen transfer is a limiting factor. The BIOSTAT® CultiBag STR 50 plus MO is a single use bioreactor, which is specially designed to fulfil the requirements of microbial cultivations.

An *Escherichia coli* fed batch cultivation was carried out to prove the suitability of this system for microbial application. A cell density of OD_{600} = 160 (DCW = 60.8 g/L) was achieved. Furthermore, the oxygen transfer of the system was sufficient meet the requirements of the cells. Based on this, *E. coli* high cell density cultivations under aerobe conditions are possible in the BIOSTAT® CultiBag STR 50 plus MO.

In a parallel cultivation performed in a stainless steel fermenter and single use bioreactor similar results were obtained. Therefore, a process transfer and scale up from the described single use system to a stainless steel fermenter or vice versa is possible.

1. Introduction

Single use systems are an innovative alternative to conventional stainless steel bioreactors. Compared to reusable systems they are highly flexible, because all connections are disposable and offer the opportunity to be adapted to the particular application. Costs are reduced by eliminating the space consuming and expensive CIP/SIP installations. The production turn around time is also reduced dramatically. The risk of cross contaminations is eliminated almost completely because every component, which is in direct product contact, is discarded after the fermentation. In combination with the reduced validation effort, this eventually results in a shorter time to market (Eibl et al., 2009).

Many different options of single use bioreactors, with pre sterilized cultivation chambers, exists on the market. They differ in terms of shape, the type of aeration and agitation. Most of these systems rely on different design principles as their stainless steel counterparts. Therefore, the process transfer and scale up from single use to reusable bioreactors is often difficult. The BIOSTAT® CultiBag STR (Sartorius Stedim Biotech) is the first and only single use bioreactor, which was engineered based on the well characterized principles of classical reusable bioreactors. They have a cylindrical cultivation chamber, impellers mounted on a rigid shaft and the aeration is carried out by a submerged sparger.

The single use bag has a height to diameter ratio of 2:1, with a convex bottom and the harvesting port at the lowest position. This bioreactor is available in the scales 50 L, 200 L, 500 L and 1000 L as a cell culture system. For the agitation the opportunity of a 6-blade-disk and 3-blade-segment impeller exists. All materials, which are in contact with the fermentation broth, are USP class VI compliant (Noack et al., 2011).

Nowadays, single use bioreactors have become widely accepted for cell cultural applications. Reports about the successful use for microbial cultivations are rare. Commonly the oxygen transfer of these systems is to low to match the demand of fast growing microorganisms.

The BIOSTAT® CultiBag STR 50 plus MO (see figure 1) is a further development of the well established STR 50 cell culture version. The stirrer speed and therefore the power input have been increased compared to the basis system. Furthermore, the aeration rates are adapted to microbial requirements. As a consequence, the oxygen transfer of this bioreactor is significantly improved. Volumetric mass transfer coefficients (k_La) above 150 h^{-1} can be achieved with this bioreactor (data Sartorius Stedim Biotech).

Figure 1: BIOSTAT® CultiBag STR 50 plus MO.

The adaption to microbial applications makes modifications necessary. To avoid disadvantage flow characteristics at higher stirrer speeds baffles are installed at the outside of the cultivation chamber (see figure 2 a). Due to the increased gas flow, needed for microbial fermentations, the humidity can cause a blockage of the exhaust gas filters. Therefore, a single use exhaust gas cooler is installed (see figure 2 b).

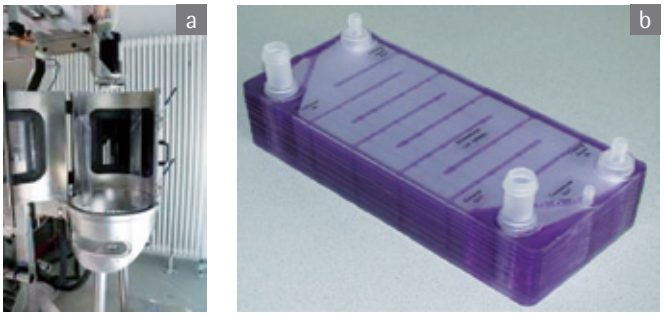

Figure 2: (a) Baffles, (b) single use exhaust gas cooler.

In this application note, we present a high cell density *Escherichia coli* cultivation in the BIOSTAT® CultiBag STR 50 plus MO. A fed batch process with an exponential increasing feeding rate was carried out. The results are compared with the established stainless steel fermenter BIOSTAT® C+.

2. Material and methods

2.1 Bacterial strain

For the cultivation *Escherichia coli* BL21 (DE3) (Novagen, Merck, Darmstadt, Germany) was applied.

2.2 Used cultivation media

2.2.1 Composition LB-medium

For the preparation of the first seed culture in 50 mL CultiFlasks LB-medium was used. It contained 20 g/L LB-medium powder (Roth). For the sterilization it was autoclaved 20 minutes at 121°C.

2.2.2 Composition defined medium

For the following cultivation steps a defined mineral salt medium was used (Riesenberg et al., 1991). It contained KH_2PO_4 (13.3 g/L), $(NH_4)_2HPO_4$ (4.0 g/L), $C_6H_8O_7$ (1.7 g/L), D+glucose (10 g/L), $MgSO_4 \cdot 7H_20$ (1.2 g/L). Additionally, a trace element solution was added to supply the medium with (final concentration) $CoCl_2 \cdot 6H_20$ (0.25 mg/L), $CuCl_2 \cdot 2H_20$ (0.15 mg/L), H_3BO_3 (0.3 mg/L), $Na_2MoO_4 \cdot 2H_20$ (0.25 mg/L), $Zn(CH_3COO)_2 \cdot 2H_20$ (0.8 mg/L), Titriplex III (0.84 mg/L), Fe(III)citrate (6 mg/L) and $MnCl_2 \cdot 2H_20$ (1.23 mg/L). The medium was sterile filtrated by a Sartopore® 2 (Sartorius Stedim Biotech) with a pore size of 0.2 µm.

2.2.3 Composition feed solution

During the cultivation in the STR 50 plus MO a feed solution, containing 770 g/L glucose, was supplied. The corresponding amount was solved in RO-water and autoclaved. Afterwards, a 400 g/L $MgSO_4 \cdot 7H_20$ stock solution was added to achieve a concentration of 19.7 g/L. The stock solution was added by sterile filter (Sartolab, Sartorius Stedim Biotech) with a pore size of 0.2 µm.

2.3 Cultivation steps

The first seed culture was prepared in 50 mL CultiFlasks (Sartorius Stedim Biotech) filled with 10 mL LB-medium (see chapter 2.2.1). For the second seed culture a 1 L Erlmeyer flask filled with 200 mL defined medium (see chapter 2.2.2) was used. The incubation of the first and second seed culture was carried out in a shaking incubator (Certomat IS, Sartorius Stedim Biotech) at 37°C, 150 rpm (orbit diameter = 50 mm).

Afterwards, the third seed culture, was cultivated in the BIOSTAT® CultiBag RM 20/50 optical system (Sartorius Stedim Biotech). For the temperature control a cooling coil was mounted. As cultivation chamber a 10 L CultiBag RM optical was used. It was filled with 5 L defined medium (see chapter 2.2.2).

The rocking angle was set to its maximal value of 10°. The aeration rate was set to a constant flow of 4 Lpm and the rocking rate to 35 min^{-1}. If necessary, pure oxygen was supplied to the system. The pO_2 was controlled to 50 % and the pH to 6.8 by the supplementation of 20 % ammonia solution. The temperature was controlled to 37 °C. The cultivation conditions were maintained by a Digital Control Unit tower (DCU, Sartorius Stedim Biotech).

For the fermentation of the main culture a BIOSTAT® CultiBag STR 50 plus MO with a double wall system was used. The temperature was controlled by the cooling unit Frigomix 2000 (Sartorius Stedim Biotech).

The used CultiBag STR 50 had the configuration 6-blade-disk impeller (bottom) and a 3-blade-segment impeller (top). For the aeration a ring sparger with 5 holes and a diameter of 0.8 mm was installed. Depending on the oxygen demand the agitation rate was varied between 250 – 370 rpm and the aeration rate between 5 – 20 Lpm. If necessary, pure oxygen was supplied. The k_La-value for these settings and bag configuration is 163 h^{-1} (data Sartorius Stedim Biotech).

To avoid vortexing effects at higher stirrer speed baffles were installed outside the cultivation chamber. Because a high humidity can cause blocking of the exhaust gas filter a single use exhaust gas cooler was installed. The initial volume was 30 L (defined medium, see chapter 2.2.2) and 10 L feed solution (see chapter 2.2.3) were added during the cultivation.

The temperature was controlled to 28 °C and the pO_2 to 20 %. By the supplementation of 20 % ammonia solution the pH was set to 6.8. The pO_2 and pH were measured during the cultivation by classical probes (Hamilton) and optic chemical sensors (PreSens). With a Digital Control Unit (DCU, Sartorius Stedim Biotech) the control of the cultivation conditions was carried out. For the comparison of the results a cultivation in a stainless steel fermenter (BIOSTAT® C+ 10-3, Sartorius Stedim Biotech) was carried out. The cultivation conditions were the same like for the STR 50 plus MO.

2.4 Materials

- BIOSTAT® CultiBag STR 50 plus MO single use bioreactor (Sartorius Stedim Biotech)
- CultiBag STR 50 disposable bioreactor bag (Sartorius Stedim Biotech) containing a 6-blade-disk impeller (bottom), a 3 blade-segment impeller and a ring sparger
- BIOSTAT® CultiBag RM 20/50 optical system (Sartorius Stedim Biotech)
- 10 L CultiBag RM optical disposable bioreactor (Sartorius Stedim Biotech)
- Flexel® 3D LevMix mixing system (Sartorius Stedim Biotech)
- BioWelder tube welder for thermoplastic tubes (Sartorius Stedim Biotech)
- BioSealer tube sealer for thermoplastic tubes (Sartorius Stedim Biotech)
- Frigomix 2000 cooling unit (Sartorius Stedim Biotech)

2.5 Fed batch cultivation

For the cultivation in the BIOSTAT® CultiBag STR 50 plus MO a fed batch strategy with exponential supplementation of the limiting substrate was used. After the consumption of the initial glucose in the medium the feed solution (see chapter 2.2.3) was supplied. The added amount of feed solution was calculated by equation 1.

Equation 1:

$$F(t) = \frac{\mu_{Set} \cdot X_0 \cdot V_0}{Y_{X/S} S_{Feed}} \exp(\mu_{Set} t)$$

$F(t)$ – flow rate feed solution [$g_{glucose}/h$]
μ_{Set} – fix value of the specific growth rate [h^{-1}]
$Y_{X/S}$ – yield coefficient [$g_{DCW}/g_{glucose}$]
X_0 – initial cell density at feed start [g_{DCW}/L]
V_0 – initial volume [L]
S_{Feed} – concentration of the feed solution [$g_{glucose}/L$]

The specific growth rate was controlled to 0.1 h^{-1}.
For the cultivation in the BIOSTAT® C+ the same μ_{Set} was used.

3. Results

After the inoculation of the systems samples of the fermentation broth were taken in an interval of one hour. To get a statement about the growth behaviour the optical density was determined at 600 nm (OD_{600}). Furthermore, the metabolism was examined by the measurement of the glucose and acetate concentration in the medium.

In figure 3 the characteristics of the OD_{600} and the specific growth rate for the STR 50 plus MO and C+ are compared. For the time prior the feed start (batch phase) the cell density increased exponential in both systems. At the end of the batch phase (t = 9 h) the cell density in the STR 50 plus MO was OD_{600} = 13.9 (DCW = 5.2 g/L). For the C+ the optical density was at the end of the batch phase 13 (DCW = 4.9 g/L). Therefore, the yield coefficient for the STR 50 plus MO was $Y_{X/S}$ = 0.52 $g_{DCW}/g_{glucose}$ and $Y_{X/S}$ = 0.49 $g_{DCW}/g_{glucose}$ for the stainless steel system, respectively. The inoculation volume for the STR 50 plus MO cultivation was 3 times higher compared to the fermentation in the C+. This results in a shorter batch phase.

After the supplementation of the feed solution (fed batch phase) the cell density further increased exponential in both systems. Because the specific growth rate was controlled to a value below μ_{max} the slope of the growth curve was lower compared to the batch phase. After approximately 39 h a cell density of OD_{600} = 162 (DCW = 61.7 g/L) was determined for the stainless steel system. In the STR 50 plus MO an optical density of 160 (DCW = 60.8 g/L) was measured.

A constant μ of 0.4 h^{-1} was determined for both systems during the batch phase (0 – 12 h for the C+, 0-9 h for the STR plus MO). This corresponds to the maximal specific growth rate at 28°C (Dreher, 2010). After the feed start μ decreased. It was possible to control the specific growth rate to 0.1 h^{-1}.

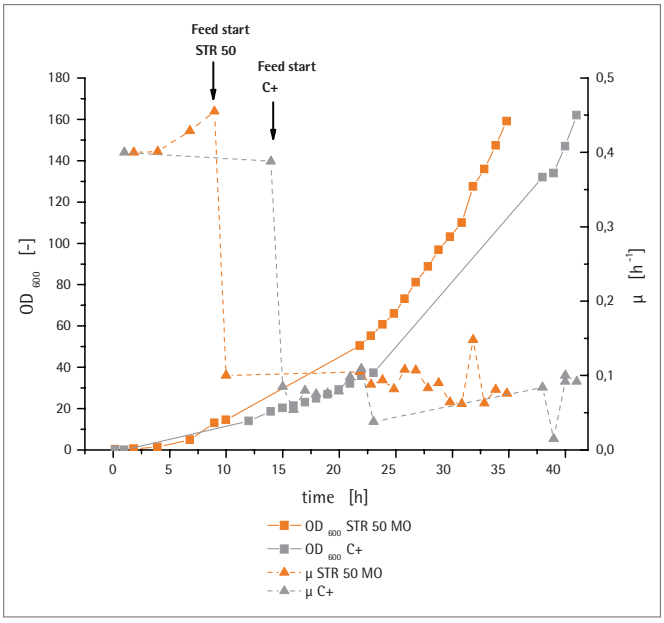

Figure 3: Growth behaviour during the *Escherichia coli* cultivations in the BIOSTAT® CultiBag STR 50 plus MO and BIOSTAT® C+. The characteristics of the OD_{600} and specific growth rate are shown.

To guarantee ideal growth behaviour it is necessary to control the cultivation conditions (see figure 4). One important factor is the pH-value. For the whole cultivation it was possible to keep the pH in the defined range around the set point for both systems.

The yield of *E. coli* is significantly higher under aerobe conditions. Therefore, a control of the dissolved oxygen concentration is advantageous. During the cultivations the pO_2 was close to or above the set point of 20 %. Based on this, aerobe cultivation conditions were guaranteed in both systems.

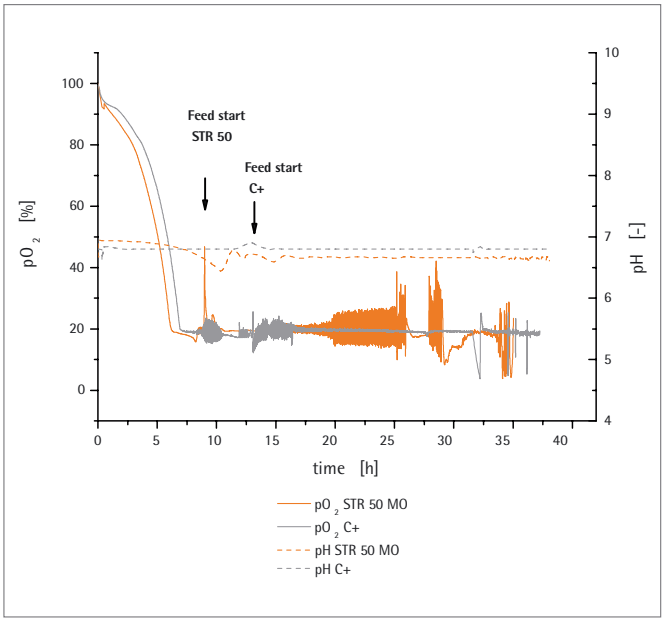

Figure 4: Cultivation conditions. The characteristics of the pH and pO_2 are shown.

For the measurement of the pH and pO_2 two different measuring principles were used. In figure 5 the measured data of the optic chemical and classical sensors are represented. The values for the pO_2 as well as the pH of both measuring principles showed only low deviations.

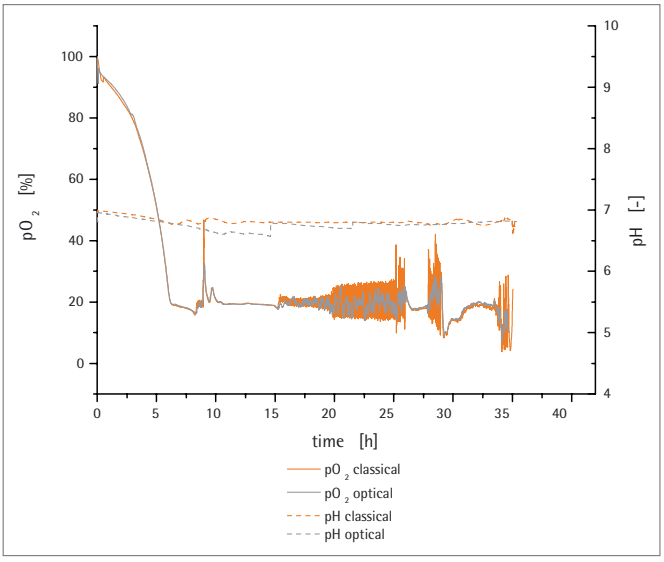

Figure 5: Comparison of the optic chemical sensors and the classical probes. The characteristics of the pH and pO_2 are shown.

To make conclusions about the metabolic activity during the cultivations the glucose and acetate concentrations were determined (see figure 6 a,b). The initial glucose concentration was 9 g/L for the STR 50 plus MO and 9.8 g/L for the C+. At the end of the batch phase the submitted glucose was consumed. During the supplementation of the feed solution no glucose was detectable. Hence, it can be assumed that the entering glucose was immediately consumed.

One metabolic product of *E. coli* is acetate, which is produced under oxygen limiting conditions or by overflow metabolism. It can have an inhibitory and at higher concentrations a toxic effect. The characteristics of the acetate concentrations are shown in figure 6 a) and b). No accumulations were observed during the cultivations. Therefore, neither overflow metabolism nor oxygen limitations occurred.

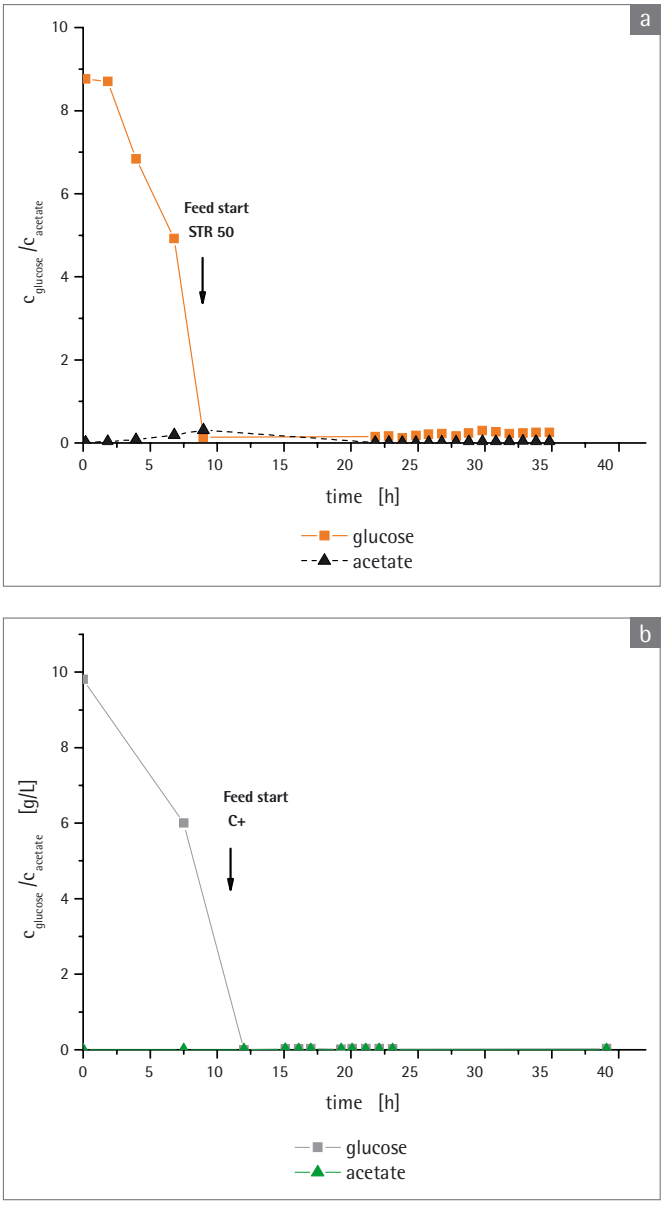

Figure 6: Analysis of the metabolism. The acetate and glucose concentrations are shown. Characteristics of the (a) BIOSTAT® CultiBag STR 50 plus MO, (b) BIOSTAT® C+.

4. Discussion

The BIOSTAT® CultiBag STR 50 plus MO is a modified version of the well established cell culture bioreactor. It is a single use bioreactor, which is engineered completely on principles known from reusable bioreactors. To examine this system for microbial suitability an *Escherichia coli* fed batch cultivation was carried out. A cell density of OD_{600} = 160 (DCW = 60.8 g/L) was achieved. This is comparable to industrial *E. coli* cultivations in stainless fermenters. Hence, the results show that microbial high cell density cultivations are possible in the BIOSTAT® CultiBag STR 50 plus MO.

During the cultivation the pO_2 was close to or above the set point. Therefore, aerobe conditions were ensured. This implies that the oxygen transfer is sufficient for microbial cultivations.

The control of the pH was possible and no accumulation of glucose or acetate occurred. Hence, the cultivation conditions were optimal for *E. coli* during the fermentation.

The measured values of the optic chemical and classical sensors were approximately similar. Based on this, both measuring principles are suitable.

To compare the results a cultivation in the stainless steel fermenter BIOSTAT® C+ was performed. No significant differences were observed between the stainless steel system and the STR 50 plus MO. Therefore, a process transfer or a scale up with the presented single use system to a stainless steel fermenter or vice versa is possible.

References

T. Dreher: Evaluation and process optimization of a single use bioreactor system. Diploma thesis, Sartorius Stedim Biotech 2010

R. Eibl, S. Werner, D. Eibl: Disposable bioreactors for plant liquid cultures at litre-scale. Eng. Life Sci. 2009, 9 No. 3, 156-164.

U. Noack, F. Verhoeye, W. Kahlert, D. de Wilde, G. Greller: Disposable stirred tank reactor BIOSTAT® CultiBag STR: Characterization and applications.

D. Riesenberg, V. Schulz, W. A. Knorre, H. -D. Pohl, D. Korz, E. A. Sanders, A. Roß, W. D.Deckwer: High cell density cultivation of *Escherichia coli* at controlled specific growth rate. Journal of Biotechnology Vol. 20, 1991:17-27

Sales and Service Contacts

For further contacts, visit www.sartorius-stedim.com

Europe

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen

Phone +49.551.308.0
Fax +49.551.308.3289

www.sartorius-stedim.com

Sartorius Stedim Systems GmbH
Schwarzenberger Weg 73–79
34212 Melsungen

Phone +49.5661.71.3400
Fax +49.5661.71.3702

www.sartorius-stedim.com

France
Sartorius Stedim Biotech S.A.
ZI Les Paluds
Avenue de Jouques – BP 1051
13781 Aubagne Cedex

Phone +33.442.845600
Fax +33.442.845619

Sartorius Stedim France SAS
ZI Les Paluds
Avenue de Jouques – CS 71058
13781 Aubagne Cedex

Phone +33.442.845600
Fax +33.442.846545

Austria
Sartorius Stedim Austria GmbH
Franzosengraben 12
A-1030 Vienna

Phone +43.1.7965763.18
Fax +43.1.796576344

Belgium
Sartorius Stedim Belgium N.V.
Leuvensesteenweg, 248/B
1800 Vilvoorde

Phone +32.2.756.06.80
Fax +32.2.756.06.81

Denmark
Sartorius Stedim Nordic A/S
Hoerskaetten 6D, 1.
DK-2630 Taastrup

Phone +45.7023.4400
Fax +45.4630.4030

Hungary
Sartorius Stedim Hungária Kft
Kagyló u. 5
2092 Budakeszi

Phone +36.23.457.227
Fax +36.23.457.147

Italy
Sartorius Stedim Italy S.p.A.
Via dell'Antella, 76/A
50012 Antella-Bagno a Ripoli (FI)

Phone +39.055.63.40.41
Fax +39.055.63.40.526

Netherlands
Sartorius Stedim Netherlands B.V.
Edisonbaan 24
3439 MN Nieuwegein

Phone +31.30.6025080
Fax +31.30.6025099

Poland
Sartorius Stedim Poland Sp. z o.o.
ul. Wrzesinska 70
62-025 Kostrzyn

Phone +48.61.647.38.40
Fax +48.61.879.25.04

Spain
Sartorius Stedim Spain SA
C/Isabel Colbrand 10,
Oficina 70
Polígono Industrial de Fuencarral
28050 Madrid

Phone +34.90.2110935
Fax +34.91.3589623

Switzerland
Sartorius Stedim Switzerland AG
Ringstr. 24 a
8317 Tagelswangen

Phone +41.52.354.36.36
Fax +41.52.354.36.46

U.K.
Sartorius Stedim UK Limited
Longmead Business Park
Blenheim Road, Epsom
Surrey KT19 9 QQ

Phone +44.1372.737159
Fax +44.1372.726171

America

USA
Sartorius Stedim North America Inc.
5 Orville Drive
Bohemia, NY 11716

Toll-Free +1.800.368.7178
Fax +1.631.254.4253

Sartorius Stedim SUS Inc.
1910 Mark Court
Concord, CA 94520

Phone +1.925.689.6650
Toll Free +1.800.914.6644
Fax +1.925.689.6988

Sartorius Stedim Systems Inc.
201 South Ingram Mill Road
Springfield, MO 65802

Phone +1.417.873.9636
Fax +1.417.873.9275

Argentina
Sartorius Argentina S.A.
Int. A. Avalos 4251
B1605ECS Munro
Buenos Aires

Phone +54.11.4721.0505
Fax +54.11.4762.2333

Brazil
Sartorius do Brasil Ltda
Av. Dom Pedro I, 241
Bairro Vila Pires
Santo André
São Paulo
Cep 09110-001

Phone +55.11.4451.6226
Fax +55.11.4451.4369

Mexico
Sartorius de México S.A. de C.V.
Circuito Circunvalación Poniente No. 149
Ciudad Satélite
53100 Naucalpan, Estado de México

Phone +52.5555.62.1102
Fax +52.5555.62.2942

Asia | Pacific

Australia
Sartorius Stedim Australia Pty. Ltd.
Unit 5, 7-11 Rodeo Drive
Dandenong South Vic 3175

Phone +61.3.8762.1800
Fax +61.3.8762.1828

China
Sartorius Stedim Beijing
Representative Office
No. 33, Yu'an Road,
Airport Industrial Zone B, Shunyi District
Beijing 101300

Phone +86.10.80426516
Fax +86.10.80426580

Sartorius Stedim Shanghai
Represantative Office
Room 618, Tower 1, German Centre,
Shanghai, PRC., 201203

Phone +86.21.28986393
Fax +86.21.28986392.11

Sartorius Stedim Guangzhou Office
Room 704, Broadway Plaza,
No. 233–234 Dong Feng West Road
Guangzhou 510180

Phone +86.20.8351.7921
Fax +86.20.8351.7931

India
Sartorius Stedim India Pvt. Ltd.
#69/2-69/3, Jakkasandra
Kunigal Road, Nelamangala Tq
Bangalore – 562 123

Phone +91.80.4350.5361
Fax +91.80.4350.5253

Japan
Sartorius Stedim Japan K.K.
KY Building, 8–11
Kita Shinagawa 1-chome
Shinagawa-ku
Tokyo 140-0001

Phone +81.3.3740.5407
Fax +81.3.3740.5406

Malaysia
Sartorius Stedim Malaysia Sdn. Bhd.
Lot L3-E-3B, Enterprise 4
Technology Park Malaysia
Bukit Jalil
57000 Kuala Lumpur

Phone +60.3.8996.0622
Fax +60.3.8996.0755

Singapore
Sartorius Stedim Singapore Pte. Ltd.
1 Science Park Road,
The Capricorn, #05-08A,
Singapore Science Park 2
Singapore 117528

Phone +65.6872.3966
Fax +65.6778.2494

Specifications subject to change without notice. Printed in Germany on paper that has been bleached without any use of chlorine. | W
Publication No.: SBT1013-e11111 · Order No.: 85032-541-32 · Ver. 11 | 2011

www.sartorius-stedim.com