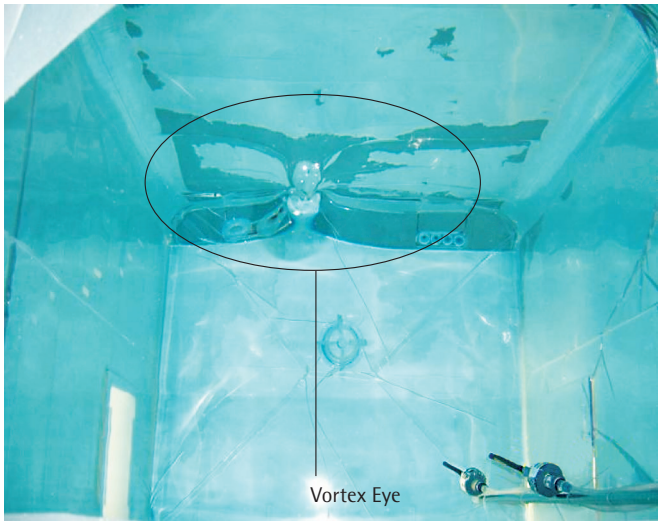
sartorius stedim biotech

1,500 L and 2,500 L buffer and media preparations with Flexel® for Magnetic Mixer

Application Note

#16
#17
#18
#19
#20

turning science into solutions



2,500 L

Executive summary

Buffer and Media preparation are an important component of many bioprocess applications. At large scale, these processes remain dominated by multiple use, stainless steel vessels. Single-use, disposable solutions have lacked adequate mixing technology at larger volumes. Development of the Flexel® for Magnetic Mixer[1] technology with compatible large scale mixing Palletank provides a unique single-use alternative to traditional stainless steel technology. This application note presents data supporting this technology to make Buffers and Media at volumes of 1,500 L and 2,500 L, as well as demonstrating liquid – liquid mixing at a volume of 2,500 L.

Introduction

Large scale Buffer and Media preparation presents a significant challenge due to the high mixing action needed to dissolve powders into solution. This application study investigates use of the Flexel® for Magnetic Mixer technology with:

- a standard 2,000 L Palletank with 2,000 L Flexel® Bag for Magnetic Mixer for buffer and media preparation at 1,500 L,
- a custom 2,500 L Palletank with 2,500 L Flexel® Bag for Magnetic Mixer for buffer and media preparation, as welll as liquid-liquid mixing at 2,500 L.

Two SAFC® ready to use powders were used in the study, 1X DPBS and Ex-Cell™ CD CHO Fusion Media. Both of these are commonly used in bioprocess applications. A high concentration salt solution was used for the liquid – liquid portion of the study. Conductivity measurement was used to determine mixing times.

Materials and methods

The list of materials and equipment used for this application is:

1. 2,000 L and 2,500 L Palletank with load cells
2. Magnetic Mixer Drive Unit (LT-DU-006-EU)
3. Eurotherm Chessell Data Logger
4. Dascor 4 – 20 mA Transmitter for conductivity
5. Sensorex conductivity probe CS200K10-TNR
6. Custom 2,500 L Flexel® Bag for Magnetic Mixer (FMB116246)
7. Standard 2,000 L Flexel® Bag for Magnetic Mixer (FMB116245)
8. Floor Scale
9. SAFC® media:
   - Ex-Cell™ CD CHO Fusion – Product Number 44075
   - Sodium Bicarbonate
   - DPBS – Product Number 56064C
10. Morton Iodized Salt
11. Conductivity Probe Calibration Standard:
    - Oakton conductivity solution, 12,880 mS/cm – P/N EW-00606-10
    - Oakton conductivity solution, 1413 mS/cm – P/N EW-00653-18

[1] This product uses Pall patented Magnetic Mixer technology. All information on patents can be found at Pall.com/patents.

Method used:

Two batches of each solution, 1X DPBS and Ex-Cell™ CD CHO Fusion media, were made; for each test volume. 1,500 L batches were made in a 2,000L Flexel® Bag for Magnetic Mixer while 2,500 L batches were made in a 2,500L Flexel® Bag for Magnetic Mixer. A conductivity probe was scaled once using 0 mS/cm in air and a 12,880 mS/cm standard prior to all experiments taking place. The sensor was mounted on a metal pole and suspended in the solutions approximately 3 feet from the bottom of the bag. Data was recorded using a Chessel Data Logger at 1 second intervals. Each batch was prepared using the following general procedure.

1. Bag was filled to 90 % of the final volume with water
2. Magnetic Mixer drive unit was set to 300 rpm.
3. Appropriate amount of DPBS or Media powder was added to the bag
4. The system was allowed to mix until no visible particles were observed and conductivity readings were consistent for 10 minutes.
5. For Media batches, the appropriate amount of Sodium Bicarbonate powder was added.
6. The system was allowed to mix until no visible particles were observed and conductivity readings were consistent for 10 minutes.
7. Bag was diluted (QS) to the final volume.
8. The system was allowed to mix until no visible particles were observed and conductivity readings were consistent for 10 minutes.

Once the final Media batch was complete, the bag was drained to a volume of 2,250 L and the liquid – liquid mixing study was performed using the following procedure.

1. A solution of 32 lbs (14.5 kg) Morton Iodized Salt in 250 L of DI water was prepared in a secondary container.
2. Magnetic Mixer drive unit was set to 300 rpm.
3. Salt solution was added to the bag as quickly as possible.
4. The system was allowed to mix until no visible particles were observed and conductivity readings were consistent for 10 minutes.

Results and discussions

The table below shows the masses for each powder added and the resulting concentrations

Solution	Final Solution Volume (L)	PBS Powder Added (kg)	CHO Media Powder Added (kg)	Bicarb Powder Added (kg)	Concen-tration PBS or CHO Media (kg/L)	Concen-tration BiCarb (kg/L)
1X DPBS	1,500	14.33			0.010	
1X DPBS	2,500	23.88			0.010	
CHO Media	1,500		30.14	1,860	0.020	0.001
CHO Media	2,500		50.23	3,130	0.020	0.001

Preliminary study has proven the linearity between conductivity and concentration of DPBS and Ex-Cell™ CD CHO Fusion as per the two following graphs:

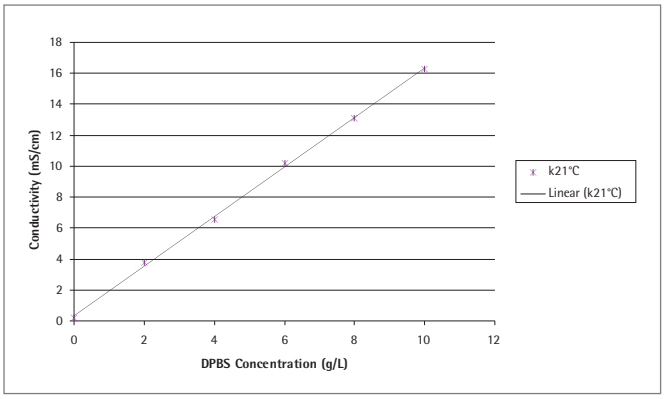


Figure 1: Conductivity vs Concentration : DPBS

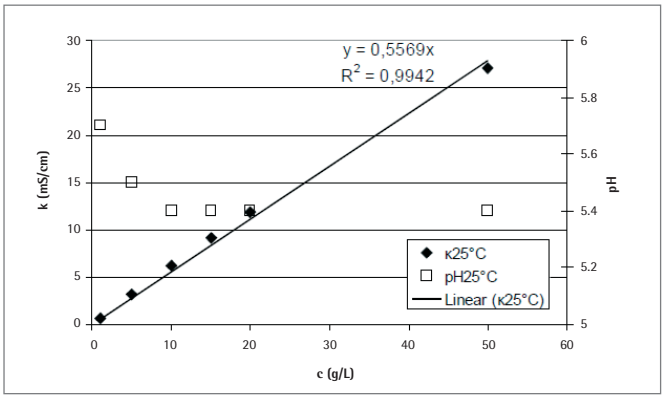


Figure 2: Conductivity and pH measurement vs Concentration of Ex-Cell™ CD CHO Fusion

The tables below show powder addition times, Mixing times, and Post QS mixing times for the four solutions made. Detailed graphs can be seen in the appendix. Powder volumes were not added as one bulk addition, but spread out over time based on visually observing dissolving efficiency. Mixing time was determined to be when the conductivity probe remained constant within a 2 % margin.

Volume (L)	DPBS Powder Addition (min)*	Mixing Time after powder addition (min)	Dilution Time (min)	Total Preparation (min)
1,500	4	9	6	19
2,500	6	13	10	29

* Multiple manual powder transfer steps

Volume (L)	CHO Powder Addition (min)*	Mixing Time after powder addition (min)	Bicar-bonate Addition + Mixing time (min)	Dilution Time (QS) (min)	Total Prepara-tion (min)
1,500	29	4	10	6	49
2,500	60	5	18	10	93

* Multiple manual powder transfer steps

Vortexes were observed at all volumes, and DPBS powder visually dissolved almost instantaneously.

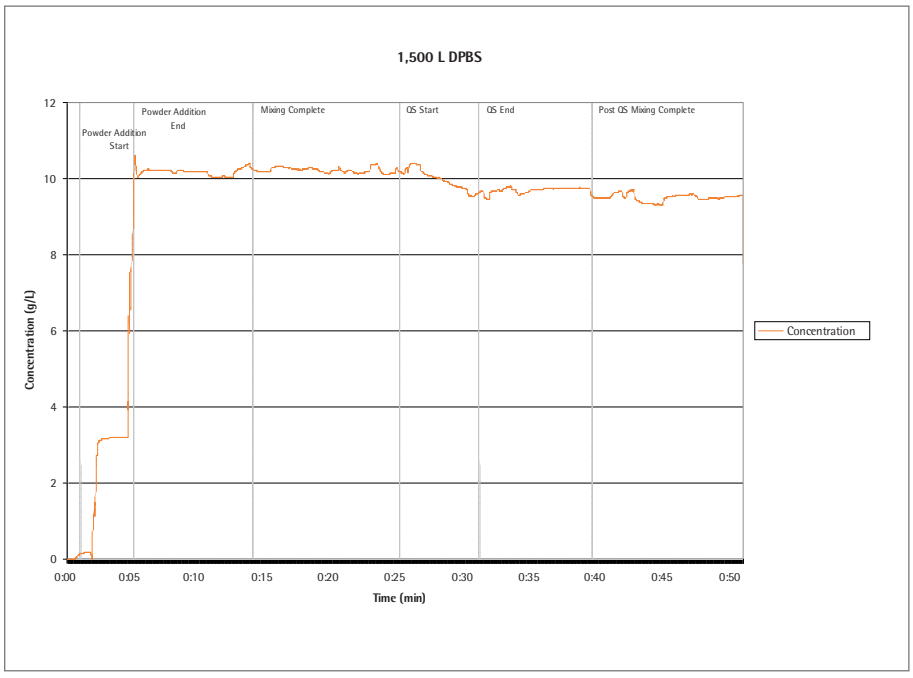
The salt solution used in the liquid – liquid portion of the study was added manually over a three minute period. Mixing time was determined to be less than 4 minutes. A detailed graph can be seen in the appendix.

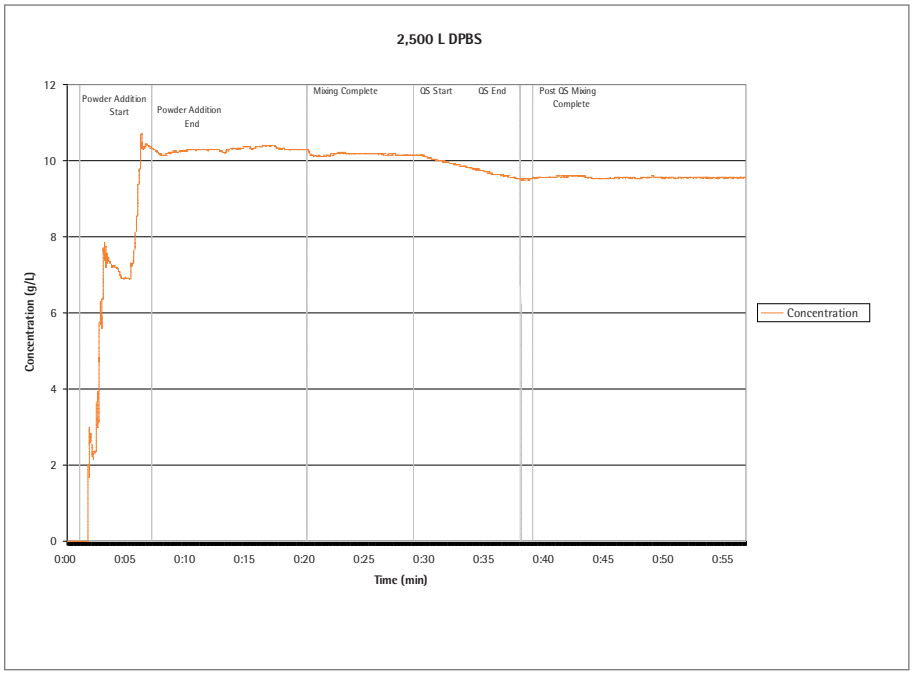
Conclusion

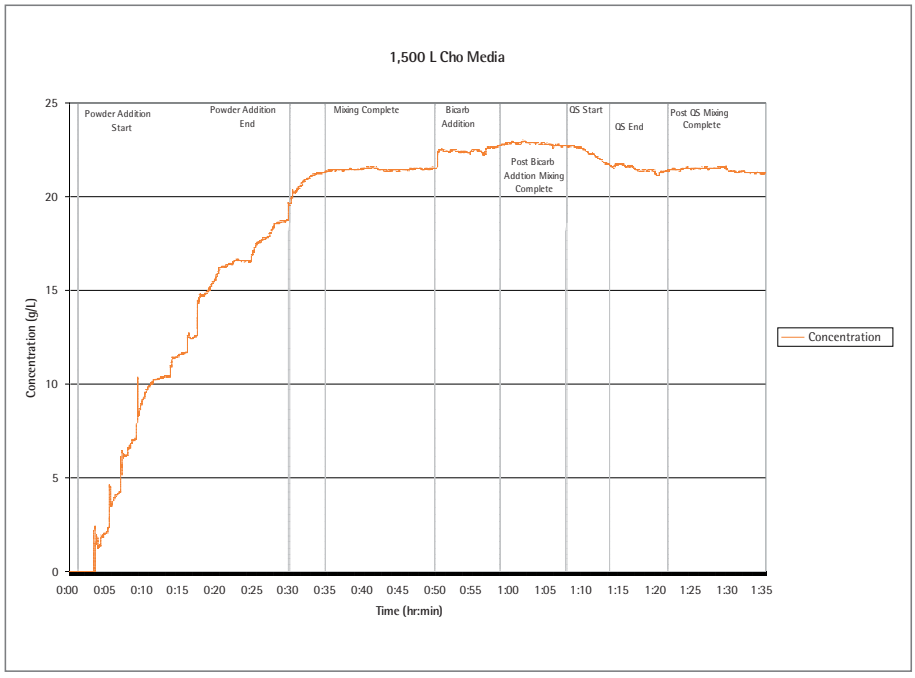
Mixing of both 1X DPBS solutions and Ex-Cell™ CD CHO Fusion media solutions using the Magnetic Mixer technology at volumes up to 2,500 L is viable. DPBS powder dissolves quickly at these volumes and complete mixing is achieved in less than 20 minutes. For Ex-Cell™ CD CHO Fusion media, mixing time at 1,500 L is less than 35 minutes, and less than 70 minutes for a 2,500 L liter batch.

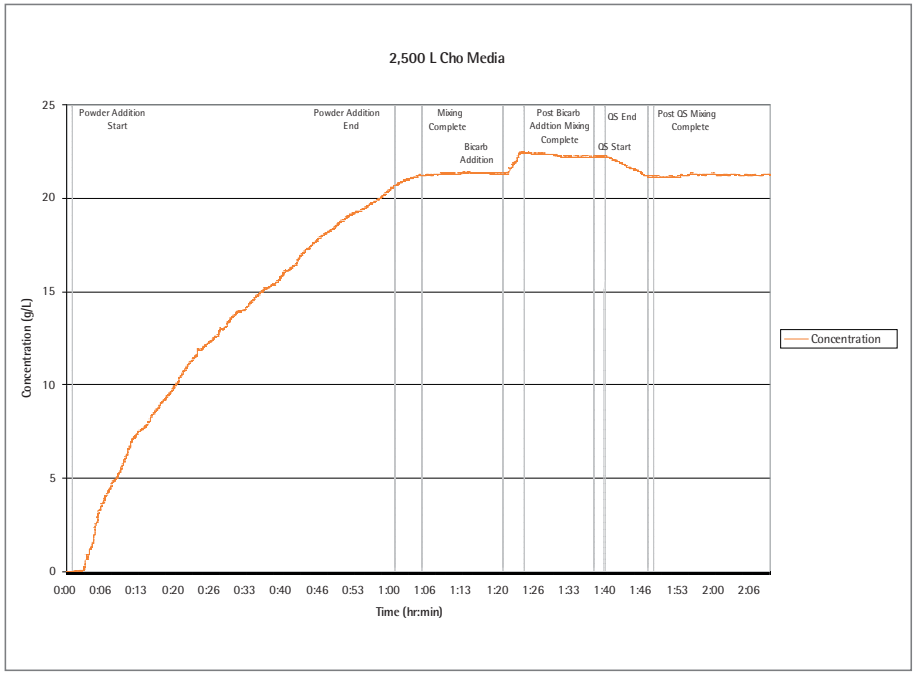
Liquid – Liquid mixing using the Magnetic Mixer Palletank system at volumes up to 2,500 L is also viable. 250 Liters of liquid were completely mixed in less than 4 minutes.

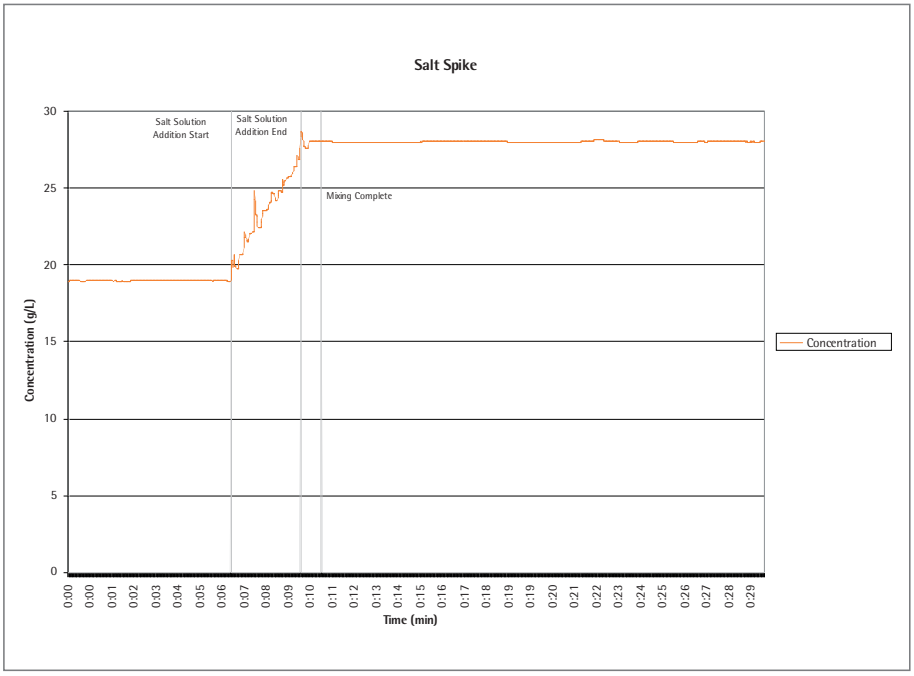
Appendix


1,500 L DPBS
Powder Addition Start
Powder Addition End
Mixing Complete
QS Start
QS End
Post QS Mixing Complete
Concentration (g/L)
Time (min)
Concentration


2,500 L DPBS
Powder Addition Start
Powder Addition End
Mixing Complete
QS Start
QS End
Post QS Mixing Complete
Concentration (g/L)
Time (min)
Concentration


1,500 L Cho Media
Powder Addition Start
Powder Addition End
Mixing Complete
Bicarb Addition
Post Bicarb Addtion Mixing Complete
QS Start
QS End
Post QS Mixing Complete
Concentration (g/L)
Time (hr:min)
Concentration


2,500 L Cho Media
Powder Addition Start
Powder Addition End
Mixing Complete
Bicarb Addition
Post Bicarb Addtion Mixing Complete
QS End
QS Start
Post QS Mixing Complete
Concentration (g/L)
Time (hr:min)
Concentration


Salt Spike
Salt Solution Addition Start
Salt Solution Addition End
Mixing Complete
Concentration (g/L)
Time (min)
Concentration
30
25
20
15
10
5
0
0:00
0:00
0:01
0:02
0:03
0:04
0:05
0:06
0:07
0:08
0:09
0:10
0:11
0:12
0:13
0:14
0:15
0:16
0:17
0:18
0:19
0:19
0:20
0:21
0:22
0:23
0:24
0:25
0:26
0:27
0:28
0:29

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

Sartorius Stedim Systems GmbH
Robert-Bosch-Strasse 5 – 7
34302 Guxhagen

Phone +49.5665.407.0

France
Sartorius Stedim FMT S.A.S.
ZI des Paluds
Avenue de Jouques – CS 91051
13781 Aubagne Cedex

Phone +33.442.845600

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

Phone +33.442.845600

Austria
Sartorius Stedim Austria GmbH
Modecenterstrasse 22
1030 Vienna

Phone +43.1.7965763.18

Belgium
Sartorius Stedim Belgium N.V.
Rue Colonel Bourg 105
1030 Bruxelles

Phone +32.2.756.06.80

Hungary
Sartorius Stedim Hungária Kft.
Kagyló u. 5
2092 Budakeszi

Phone +36.23.457.227

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

Phone +39.055.63.40.41

Netherlands
Sartorius Stedim Netherlands B.V.

Phone +31.30.60.25.080

filtratie.nederland@sartorius-stedim.com

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

Phone +48.61.647.38.40

Russian Federation
LLC "Sartorius Stedim RUS"
Vasilyevsky Island
5th line 70, Lit. A
199178 St. Petersburg

Phone +7.812.327.53.27

Spain
Sartorius Stedim Spain, S.A.U.
Avda. de la Industria, 32
Edificio PAYMA
28108 Alcobendas (Madrid)

Phone +34.913.586.098

Switzerland
Sartorius Stedim Switzerland AG
Ringstrasse 24 a
8317 Tagelswangen

Phone +41.52.354.36.36

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

Phone +44.1372.737159

Ukraine
LLC "Sartorius Stedim RUS"
Post Box 440 "B"
01001 Kiev, Ukraine

Phone +380.44.411.4918

Americas

USA
Sartorius Stedim North America Inc.
5 Orville Drive, Suite 200
Bohemia, NY 11716

Toll-Free +1.800.368.7178

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

Phone +54.11.4721.0505

Brazil
Sartorius do Brasil Ltda
Avenida Senador Vergueiro 2962
São Bernardo do Campo
CEP 09600-000 - SP- Brasil

Phone +55.11.4362.8900

Mexico
Sartorius de México, S.A. de C.V.
Libramiento Norte de Tepotzotlan s/n,
Colonia Barrio Tlacateco,
Municipio de Tepotzotlan,
Estado de México,
C.P. 54605

Phone +52.55.5562.1102

leadsmex@sartorius.com

Peru
Sartorius Peru S.A.C.
Avenue Alberto del Campo 411
Floor 12 – The Office
15076 – San Isidro, Lima

Phone +51.1.441 0158

Asia | Pacific

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

Phone +61.3.8762.1800

China
Sartorius Stedim (Shanghai)
Trading Co., Ltd.
3rd Floor, North Wing, Tower 1
No. 4560 Jinke Road
Zhangjiang Hi-Tech Park
Pudong District
Shanghai 201210, P.R. China

Phone +86.21.6878.2300

Sartorius Stedim (Shanghai)
Trading Co., Ltd.
Beijing Branch Office
No. 33 Yu'an Road
Airport Industrial Park Zone B
Shunyi District, Beijing 101300

Phone +86.10.8042.6501

Sartorius Stedim (Shanghai)
Trading Co., Ltd.
Guangzhou Branch Office
Room 1105
Xing Guang Ying Jing Building
No. 119, Shui Yin Road
Yue Xiu District, Guangzhou 510075

Phone +86.20.3836.4193

India
Sartorius Stedim India Pvt. Ltd.
#69/2-69/3, NH 48, Jakkasandra
Nelamangala Tq
562 123 Bangalore, India

Phone +91.80.4350.5250

Japan
Sartorius Stedim Japan K.K.
4th Fl., Daiwa Shinagawa North Bldg.
8-11, Kita-Shinagawa 1-chome
Shinagawa-ku, Tokyo, 140-0001 Japan

Phone +81.3.4331.4300

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

Phone +60.3.8996.0622

Singapore
Sartorius Stedim Singapore Pte. Ltd.
10 Science Park Rd
The Alpha #02-13/14
Singapore Science Park II
Singapore 117684

Phone +65.6872.3966

South Korea
Sartorius Korea Biotech Co., Ltd.
8th Floor, Solid Space B/D,
PanGyoYeok-Ro 220, BunDang-Gu
SeongNam-Si, GyeongGi-Do, 463-400

Phone +82.31.622.5700

www.sartorius-stedim.com

Publication No.: SL-1084-e180207 · Order No.: 85032-540-20 · Ver. 02 | 2018