sartorius stedim biotech

UniSart® 3D nitro slide for protein microarrays

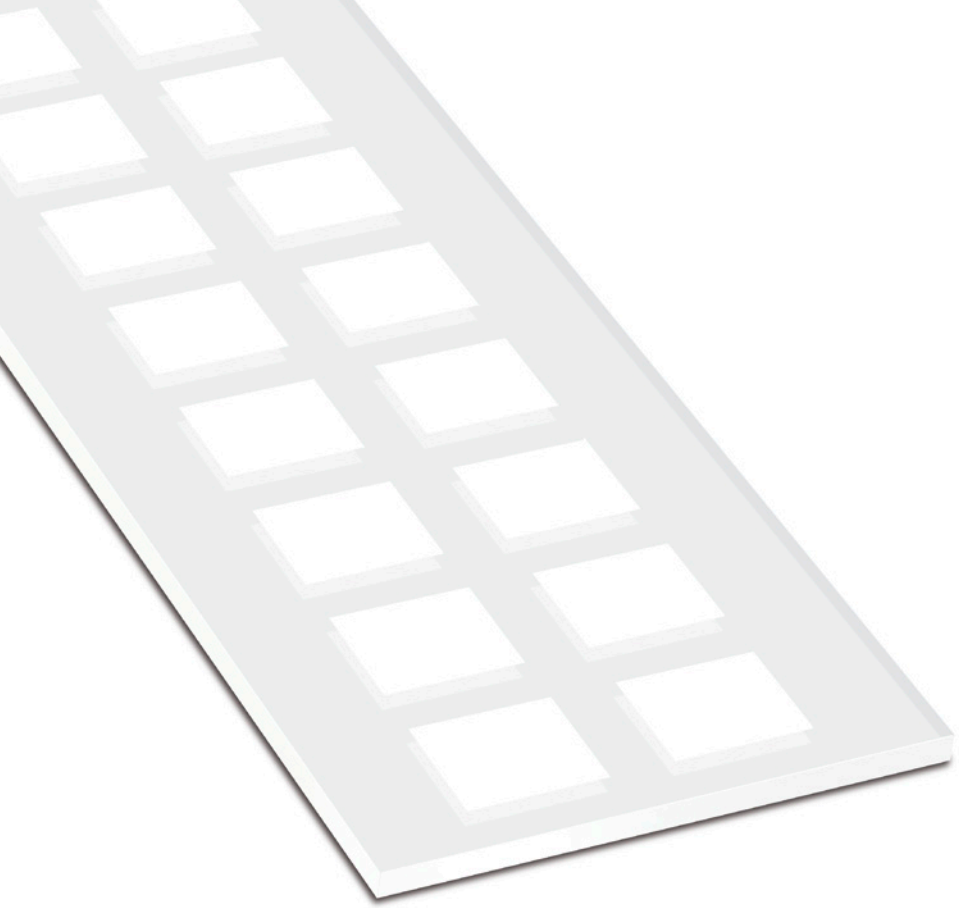
turning science into solutions

Introduction

Protein arrays or protein biochips have become an indispensable tool for many proteomic applications as well as for new multiparameter tests in clinical diagnostics. The multiplicity of protein spots, displayed in an intricate pattern on the slide surface, allows to look at various interactions simultaneously. Therefore, protein arrays, used for example in drug or antibody cross reactivity screening, considerably accelerate the quest for new drug targets and disease markers.

Modified glass surfaces have become the most widely used substrate for DNA arrays. However, proteins are quite different from nucleic acids. Proteins are heterogeneous with limited stability whereas DNA is uniform and stable. Proteins easily lose their activity through denaturation and dehydration in contrast to DNA that keeps its activity even when denatured. Signal amplification methods are readily available for DNA sequences while limited amplification options are valid for protein. All these differences support the need for a different solid substrate for protein versus DNA arrays. Today, nitrocellulose membrane is the best known surface for protein arrays.

For the new generation of protein arrays, Sartorius has developed new glass slides coated with a thin microporous nitrocellulose membrane. Widely used in electrophoresis blotting techniques and rapid diagnostic immunoassays, the classic nitrocellulose membranes needed to be reengineered to better fit the protein array applications.

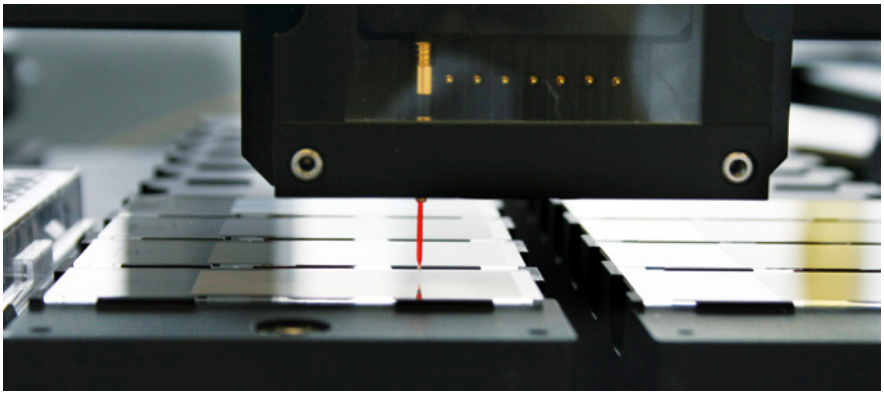


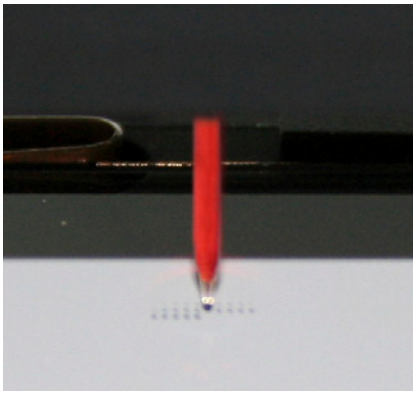
Glass slide
The glass used for the UniSart® slide is a high quality borosilicate glass slide. It has been further optimized to promote a perfect attachment of the nitrocellulose coating. When the pad format permits it, a 128 barcode label can be included on each slide for ease of identification and reliable tracking. Slide dimensions are the industrial standard in order to fit with existing instruments used by the micro-array community.

Nitrocellulose membrane pad
Due to its strong affinity for proteins, NC membranes have been used for decades in many applications like blotting and lateral flow immunoassays. Hence, NC membranes have become the reference substrate for protein binding. The unique surface characteristics of the NC membranes enable an immediate binding of proteins through electrostatic and hydrophobic interactions. This binding is not reversible under standard conditions and stabilizes proteins in their functional natural activity state. Because the 3-dimensional microporous structure offers a large internal surface area, a much bigger amount of proteins can be fixed compared to standard 2-dimensional surfaces like glass. The 3D NC membrane covering the UniSart® slides has been further optimized to show:

- An increased signal to noise ratio, even at very low protein concentrations
- A very low background of the native array slide
- A perfect spot morphology

Description
The UniSart® slide is a special glass slide coated with a thin microporous nitrocellulose (NC) membrane. The borosilicate glass slide has been especially selected for its exceptional characteristics including, flatness, hardness and fluorescence background. The nitrocellulose pad on the slide is a 3-dimensional thin microporous membrane.





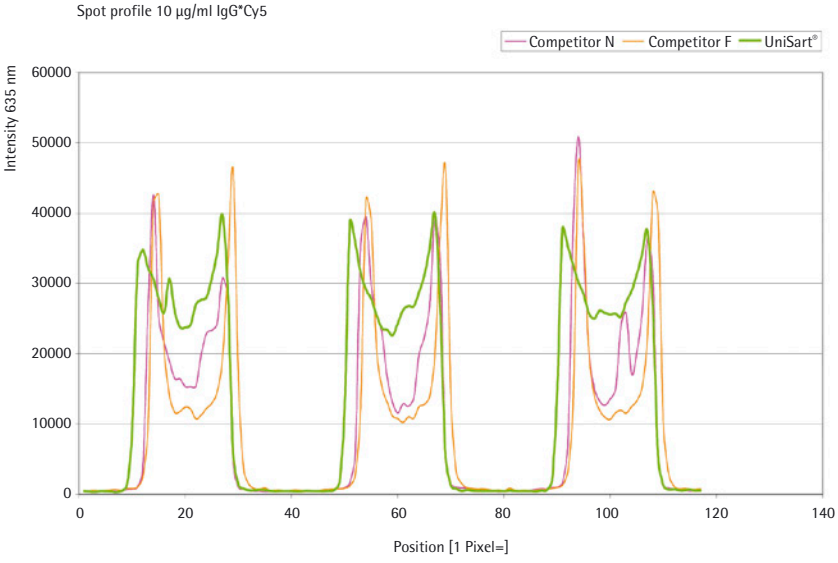
Low background surface
The surface and thickness of our white membrane pads have been designed to generate the lowest background. Standard nitrocellulose membrane usually scatters laser light and generates high fluorescence background (especially in blue wavelength). The new UniSart® slide with optimized nitrocellulose polymers and additives generate much less background compared with other white substrates.

Smooth surface
The smooth but hard surface of the UniSart® membrane allows using all type of microarray spotters. Even split pins that may generate deep membrane damages and non-uniform spot sizes due to fast membrane capillary absorption, can be used with the UniSart® slide. Nevertheless, to benefit most of the unique membrane structure, the use of non-contact spotter is of an advantage.

Precise membrane pad
The Unisart® membrane exhibits the same characteristics all over the pad including near the edges. Consequently, the whole area of the membrane pad can be used, generating better yields for protein arrayers as well as allowing for optimal hybridization and washing conditions.

Consistency
It is the most important parameter for protein array manufacturers and users. Since the first industrial production of membrane in the world started at Sartorius in 1929, we have developed a unique know-how, especially with nitrocellulose. This know-how along with new specialized manufacturing equipment for slides guarantee minimal intra- and inter-lot variability for both membrane surface and structure. Consequently, customers using UniSart® slides will experience a very high reproducibility in their experiments that will significantly reduce assay developing costs and improve final product quality.

Quality
A certificate of quality is delivered with every UniSart® slide batch. Every single UniSart® slide is 100% inspected under backlight for surface defects, stains or dust as guaranteed in the certificate.



Spot shape with different membrane slides

UniSart® 3D Nitro
has been designed to be the best substrate for protein array applications like:

– Direct protein array

– Reverse phase protein array

Direct protein array
Direct protein array, also called forward capture arrays, are slides having tiny amounts (picogram range) of purified known proteins spotted in an array format. More than 15 000 active spots can be printed. Following contact with a defined sample, a simultaneous reaction of the various components of the sample can take place with all the individual spotted molecules. Specific antibodies are commonly used as capture molecules but antigens can be spotted directly as well to detect autoantibodies in serum, for example. Peptides, aptamers, nucleic acids and enzymes may all act as alternative capture molecules. Taken together, a wide variety of protein–protein as well as protein-alternative binding partner interactions can be investigated on a protein microarray format. This interaction is then visualised and quantified with the use of classical immunostaining tools including enzyme-coupled or fluorescent labeled antibody.

Reverse phase protein array
In a reverse phase protein microarray, a multiple micro volume of cell lysate, plasma or body fluids are spotted onto the slide. The reverse phase array is then incubated against one single specific marker, usually a high quality validated antibody.

This protein array is designed as microscale dot-blot platform that allows for quantitative measurement of protein expression levels and/or post-translational modification in a large number of biological samples simultaneously. One reverse phase slide can accommodate several hundreds to thousands of samples that are printed in series of replicates. Detection is performed using specific combinations of primary and secondary antibodies. Because of the small amount of protein available, a signal amplification step is commonly introduced. The intensity of the generated chemiluminescent, fluorescent or colorimetric signals is then quantified.

Standard protocol

With and without an incubation chamber
All standard incubation chambers already available can be used with the UniSart® 3D Nitro slides.

Autostainer equipment can be used for incubation and washing steps of the slide. During longer incubation period, special care should be taken to avoid drying of the slide. The installation of a small humidifier inside the equipment and/or positioning of a hydrophobic ring around the pads through available pens or seals are recommended measures to prevent drying of the slides.

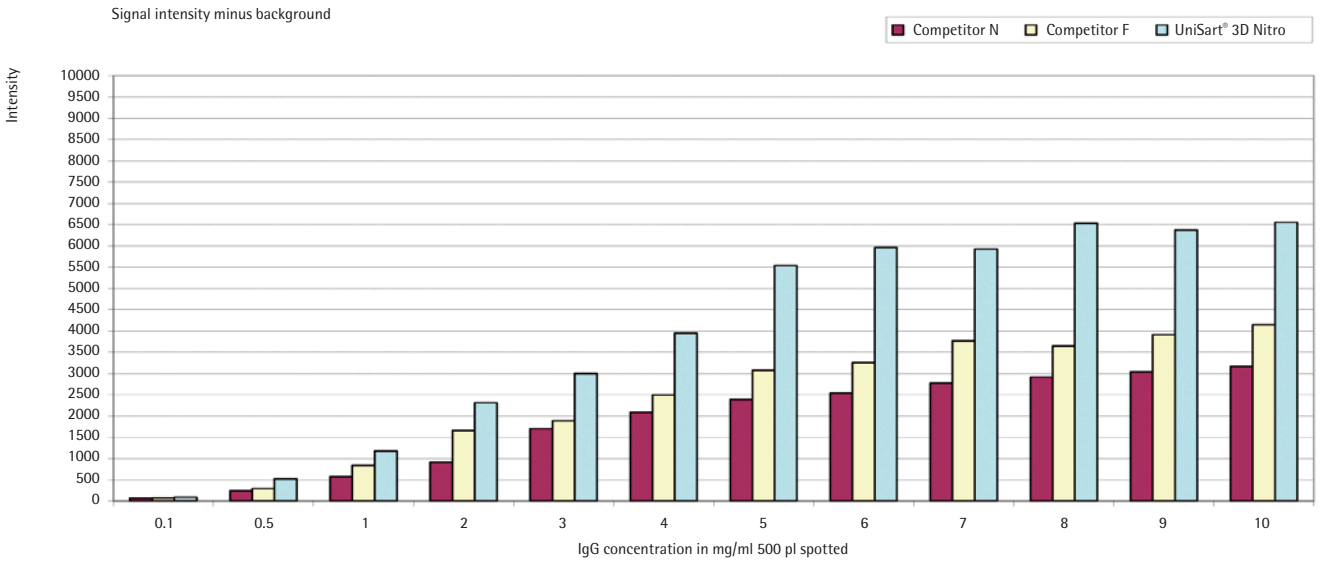
Direct protein array
Every assay is variable and is highly dependent on the specificity of the antibodies used. The protocol here included should be taken as a general guideline of the steps involved.

Antibody sandwich assay:

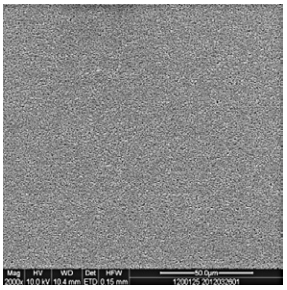
- Antibody (10-500 µg/ml) is diluted in spotting buffer (e.g.PBS, Trehalose 0.5%)
- Spotting is performed with a glass nozzle (non-contact)
- Fixation of proteins by drying 15 min at 37°C
- Blocking 1 hr at RT° with gentle shaking in PBS-0.1% Tween (PBS-T) and 2% BSA. Initial wetting of the whole nitrocellulose surface should be done carefully. Attention also needs to be given so that the slide remains wet throughout all steps.
- Incubation with biological sample in a buffer containing protease inhibitors (2 hrs/RT° – OV 4°C).
- 3 × 15 min wash in incubation buffer.
- Incubation with primary antibody OV at 4°C in PBS-T/2% BSA.
- 3 × 15 min wash in PBS-T.
- Incubation with fluorescent-labeled secondary antibody 2 hrs at RT° in PBS-T
- 3 × 15 min wash in PBS-T
- Short dip in distilled water
- Drying of slide and scan for signal intensity quantification

Reverse phase protein array
Similar to "Direct protein array", each assay is unique and the specific conditions of a given system should be tested individually. Here described, is one classical example.

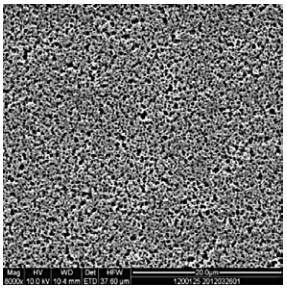
- Biological samples are lysed in lysis buffer (e.g. Laemmli buffer) containing protease inhibitors.
- Samples (1 mg/ml) are spotted with contact spotter (e.g. solid pin) and dried overnight.
- Because an amplification signal step is essential for reverse phase application, specific blocking steps (e.g. avidin/biotin block, peroxidase block) have to be performed in addition to protein block.
- Slides are then incubated in Tris buffer | Tween 0.1% (TBS-T), 5% BSA for protein block.
- Incubation with primary antibody OV at 4°C in TBS-T, 5% BSA.
- 3 × 15 min wash in TBS-T.
- Incubation with secondary antibody 1 hr at RT° in TBS-T, 5% BSA
- 3 × 15 min wash in TBS-T
- Amplification steps according to the system used (e.g. tyramide amplification) including generous wash steps
- Short dip in distilled water
- Drying of slide and scan for signal intensity quantification



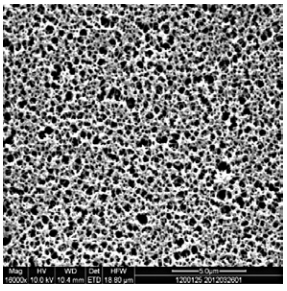
Specifications and characteristics



Scan electron microscope
of the nitrocellulose layer
@x2 000 magnification



Scan electron microscope
of the nitrocellulose layer
@x8 000 magnification



Scan electron microscope
of the nitrocellulose layer
@x16 000 magnification

Glass slide

Material	borosilicate glass
Dimension: Thickness specification	1 mm +/- 50 µm
Length	75.6 mm +/- 50 µm
Width	25.0 mm +/- 50 µm

Membrane (white)
(according to our standard test methods)

Material	cellulose nitrate polymers
Thickness	12.5 µm +/- 2.5 µm
Wettability (with test solution)	< 180 secs
Gloss (angle 60°)	50% to 60%
Background	uniform scan (at 532 nm and 635 nm)

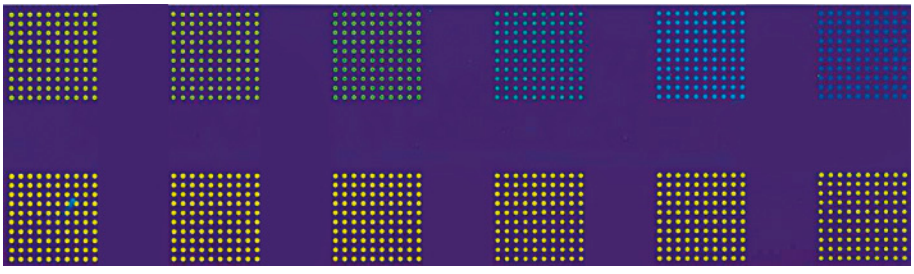
Pad dimension

One pad slide	21 mm +/- 0.2 mm on 51 mm +/- 0.2 mm
2 pads slide	20 mm +/- 0.2 mm on 20 mm +/- 0.2 mm
Shelf life	one year from manufacturing date

Ordering information

Product number	**description**
2UNY0GW051021M1G	UniSart® 3D Nitro slide one pad 51 mm × 21 mm White membrane, box of 25 slides
2UNY2GW020020M2G	UniSart® 3D Nitro slide two pads 20 mm × 20 mm White NC membrane, box of 25 slides
2UNY2GW00600616G	UniSart® 3D Nitro slide 16 pads 6 mm × 6 mm White NC membrane, box of 25 slides

How to use
Contact us for the most recommended protocols for spotting, incubating and reading of the UniSart® slides send us a mail at: unisart@sartorius-stedim.com



Scan of spotted UniSart® slide 3D Nitro slide

Background scan of UniSart® slides on Genepix scanner at 532 nm (green), 33% laser power, PMT gain 500

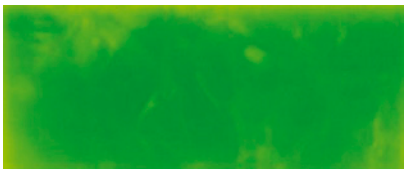
UniSart® 3D Nitro slide

Competitor N

Competitor F

Background scan at 635 nm (red), 33% laser power, PMT gain 700

UniSart® 3D Nitro slide



Competitor N

Competitor F

Low High

Sales and Service Contacts

For further contacts, visit www.sartorius-stedim.com
For further information on diagnostics e-mail us at unisart@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

unisart@sartorius-stedim.com
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

Russian Federation
OOO "Sartorius ICR"
Rasstannaya str. 2, b.2, lit.A, p/b 134
192007, Saint-Petersburg

Phone +7.812.6100821
Fax +7.812.6100821

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

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

BTChina@sartorius-stedim.com

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

Phone +86.21.28986393
Fax +86.21.28986392.11

BTChina@sartorius-stedim.com

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

BTChina@sartorius-stedim.com

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

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
Fax +82.31.622.5799

www.sartorius-stedim.com

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