

4Cell[®] CHO Media and Feed Portfolio

For Protein Production
Using CHO Cell Lines



Product Information

Sartorius' CHO media portfolio was developed for the cultivation of Chinese Hamster Ovary (CHO) and other mammalian cell lines. CHO Cells can be used for both stable and transient expression of recombinant proteins.

Our CHO media portfolio now includes 7 media formulations and 6 companion feeds suitable for the most common CHO cell lines, in batch, fed batch and perfusion cultures.

The CHO cell culture media and feeds support highly efficient protein expression and individual characteristics of different clones. It helps reduce process optimization timelines, increase cell density, and produce high titers.

Features and Benefits

- Broad choice of products accelerates media development
- Sartorius' global manufacturing network guarantees supply
- 100% chemically defined and animal component-, serum-, and hydrolysate-free to enhance reproducibility
- Customizable as well as ready-to-use, off-the-shelf kits offer flexibility
- New 4Cell[®] SmartCHO and 4Cell[®] SmartCHOpe media and feeds with high filterability and solubility attributes

Relevant Applications

- Monoclonal antibodies (mAbs) and biosimilars
- Recombinant and fusion proteins
- Standard and intensified processes
- Transient and stable clones

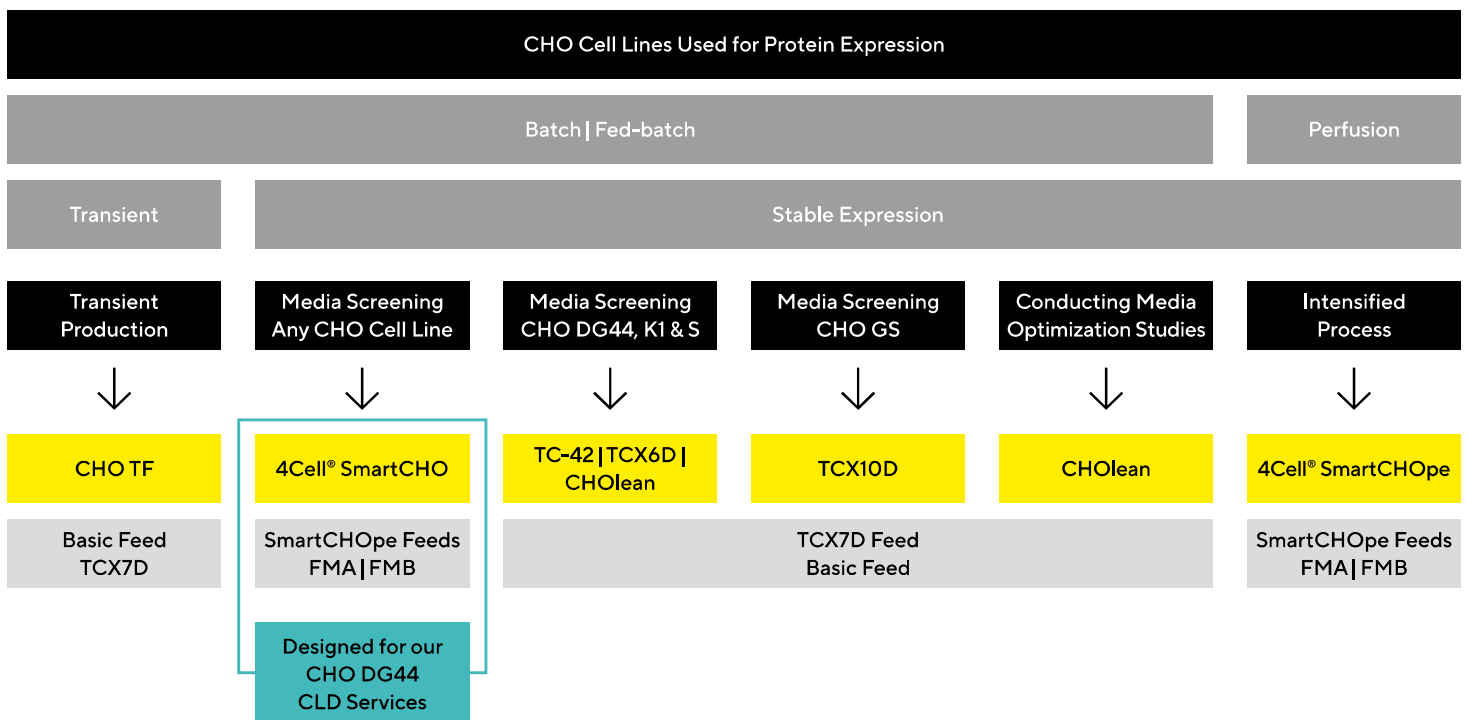
Relevant Process Steps

- **Cell Line Development and Cell Banking**
All-in-one solutions for thawing, adaptation, growth, transfection, and protein production, eliminating the need for several media types.
- **Process Development**
Using our media with the Ambr® system enables an optimized and predictive process that translates into large-scale, high-yield biomanufacturing. CHOlean media, in particular, is ideal for media optimization studies.
- **Biotherapeutics Manufacturing**
Suitable for batch, fed-batch, and perfusion processes.
- **Seed Train Culture**
Media supports stable growth and viability in high-density cultures.

Simplifying Your Decision

The optimal combination of cell culture medium and feed for your application will be influenced by your use case, cells, and production method (Figure 1).

Figure 1: A Decision Tree to Simplify Your CHO Media Selection Process.



Application

Different media formulation strategies have been used to cultivate cells in vitro for high expression of recombinant proteins. The bioprocesses employed in recombinant protein production are under strict regulatory control for safety purposes, with the use of animal-derived components in the media representing the biggest concern. With our chemically defined and animal component-free formulation, bioprocesses using our media will avoid these regulatory hurdles.

Sartorius' media portfolio supports the cultivation of CHO cells, now the most widely used cell type in the production of biomolecules. Our CHO media are designed and optimized to support the high-density suspension culture of a variety of CHO cell subtypes. Each CHO medium and its feeds have been designed for the specific use of a CHO cell expression system in suspension culture but may also be suitable for other CHO cell types (Figure 1).

Media screening can be time-consuming but finding the right formulation and feed that maximizes the potential of your cell line is necessary to promote robust growth performance and high product yields. Our comprehensive CHO media portfolio can accelerate media development and increases the chances of finding the best media for each unique application. We recommend that users perform a benchmark study to identify the most compatible media and feed for their cell line and application.



Performance

4Cell® SmartCHO – Advanced, High-Performing CHO Media

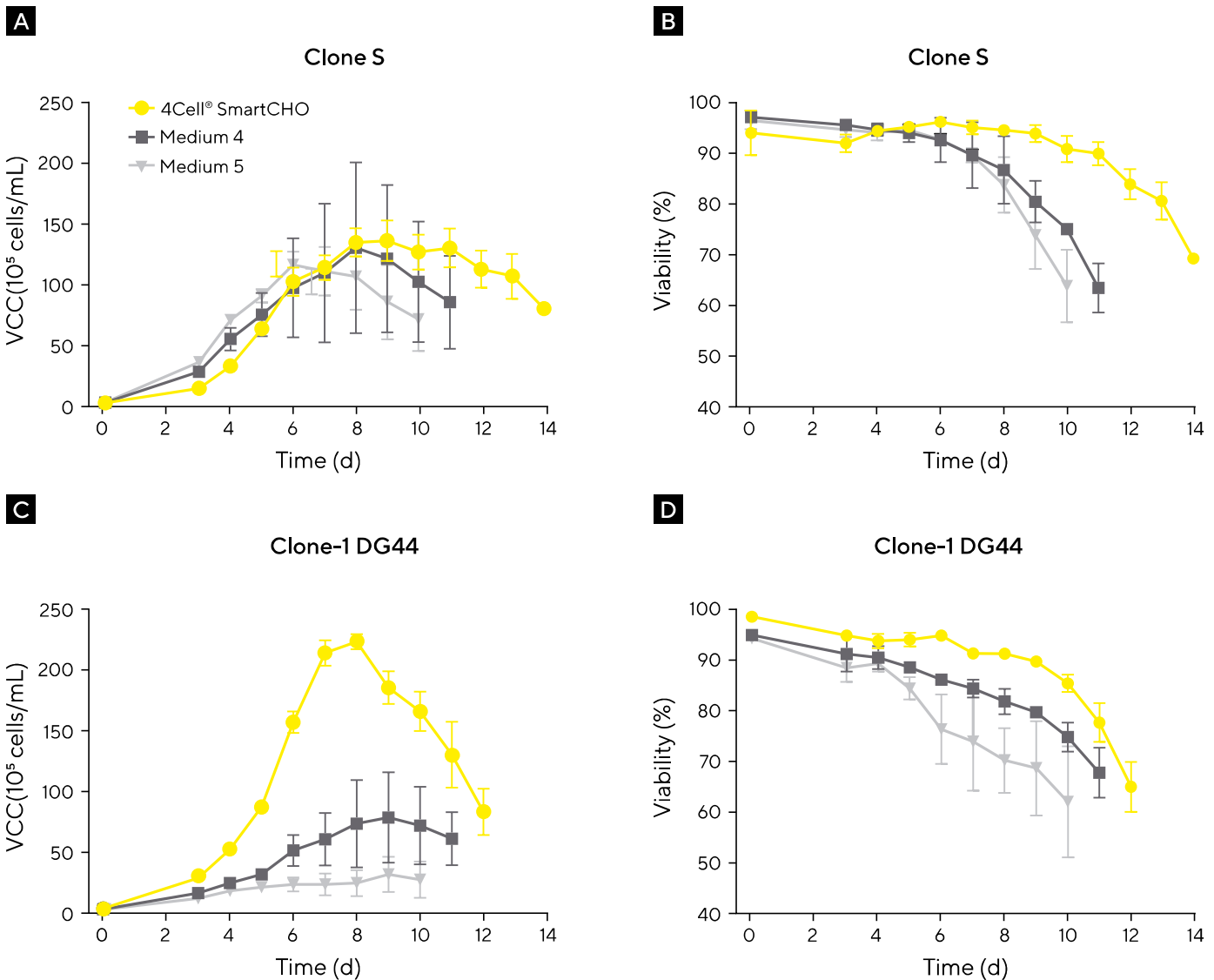
With demonstrated robust performance in fed-batch applications and ease of transfer to perfusion processes, 4Cell® SmartCHO is a go-to-choice media for any CHO cell line.

4Cell® SmartCHO media and feed system delivers robust growth and high productivity performance for CHO cell lines. Although developed with a focus on CHO DG44 cells, the basal media and feeds have been tested and found to support growth across a broad range of CHO cell lines and product formats, including mAbs, bi-specifics, and Fc fusion proteins.

We conducted a benchmarking study to evaluate the fed-batch culture performance of 4Cell® SmartCHO media and feeds compared to selected commercial media (Figure 2–8) using CHO-DG44 cell lines. In addition, we also tested its performance with CHO-S, CHO-K1, and CHO-GS clones, all cultured in the Ambr® 15.

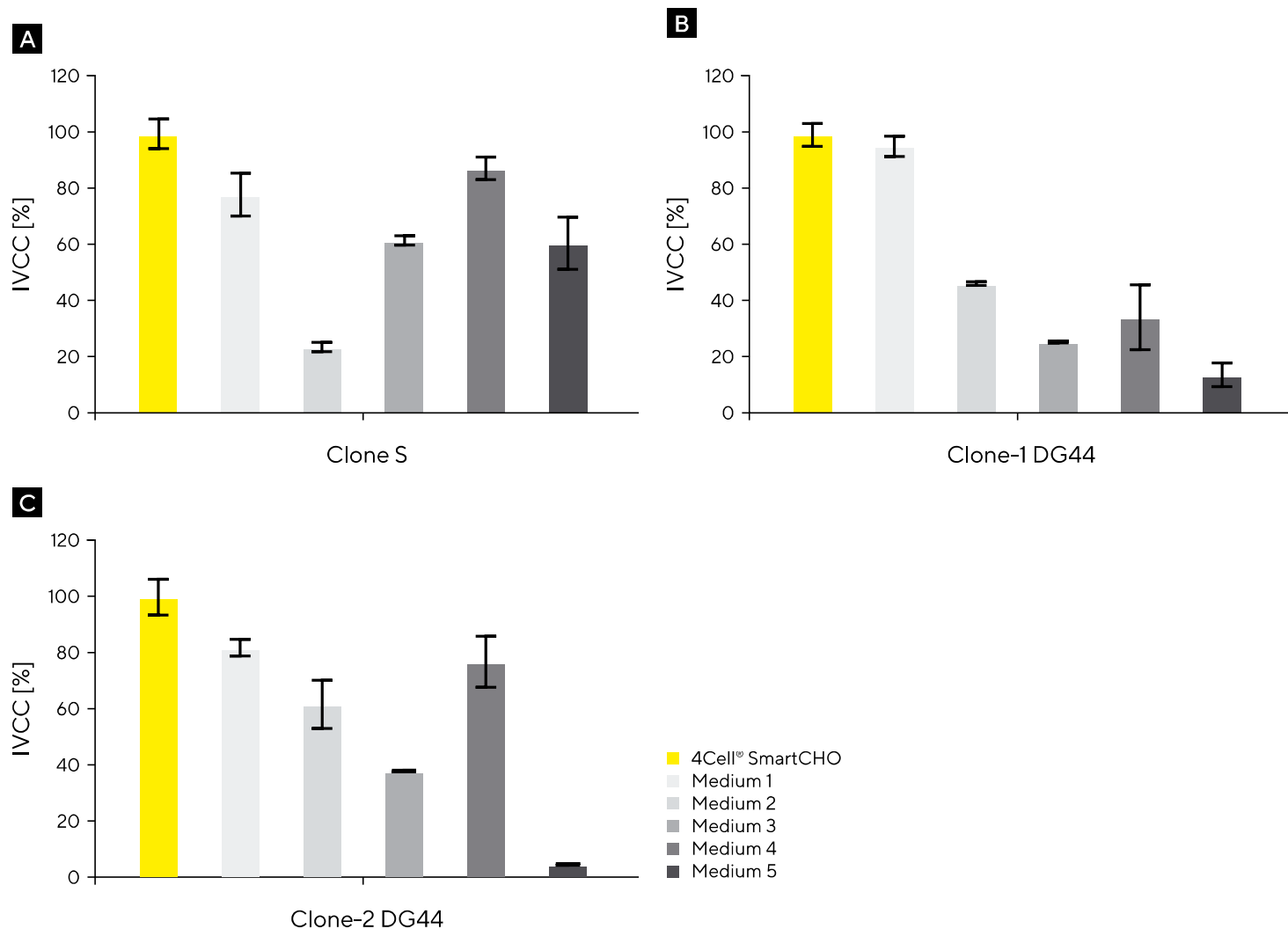
4Cell® SmartCHO media support the robust growth of CHO clones, performing as well as or better than commercial media (Figure 2 and 3).

Figure 2: Growth and Viability Profiles of Two CHO Clones in 4Cell® SmartCHO vs. Two Representative Commercial Media.



Note. (A & B) CHO-S and (C & D) CHO-DG44 cells. Cells were adapted to the media for four weeks prior to testing. (A & C) Viable cell count (VCC) and (B & D) percentage viability was measured daily for 14 days.

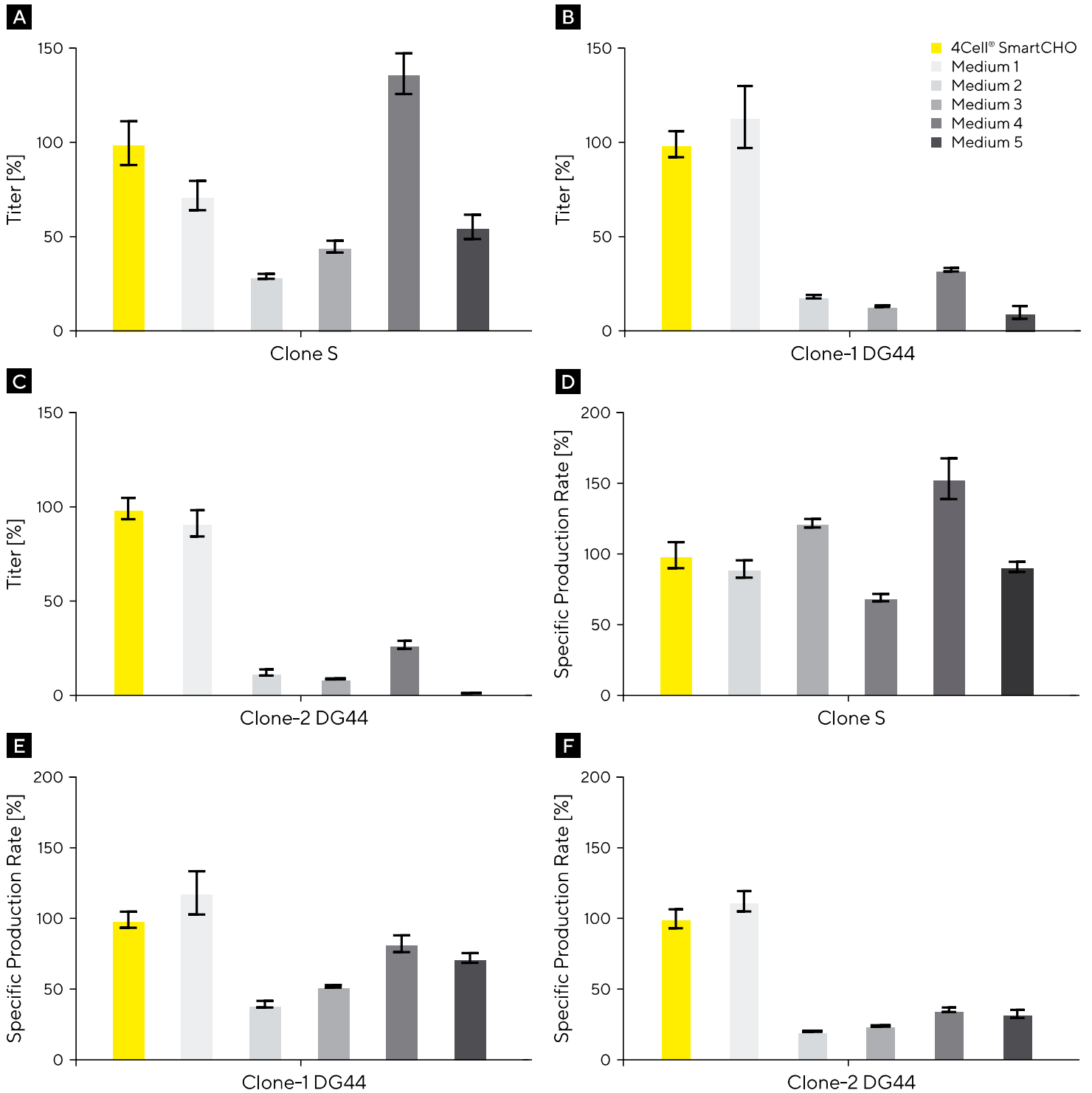
Figure 3: Relative Growth of Three CHO Clones in Cultured in 4Cell® SmartCHO vs. Five Commercial Media.



Note. (A) CHO-S, (B) Clone 1 CHO-DG44, (C) Clone 2 CHO-DG44. Relative growth is shown as integrated viable cell concentration (IVCC).

The specific productivity of CHO cells varies significantly across clones and media, highlighting the need to screen multiple formulations, feeds, and parameters depending on your cell line and application (Figure 4).

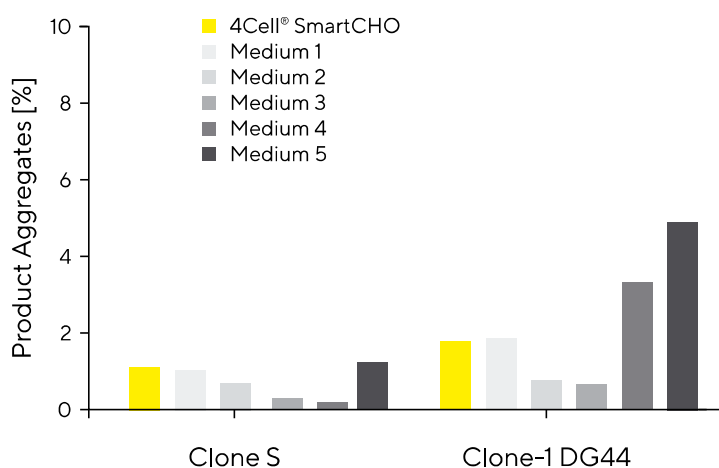
Figure 4: Titer and Specific Productivity of CHO Clones Cultured in 4Cell® SmartCHO vs. Commercial Media.



Note. (A-C) Titer [%]; (D-F) Specific Productivity Rate [%]. (A+D) CHO-S, (B+E) Clone 1 CHO-DG44, (C+F) Clone 2 CHO-DG44 were cultured in 4Cell® SmartCHO and five commercial media.

Cell culture media have a significant impact on product quality attributes. Size-exclusion chromatography reveals that the CHO-S clone maintains low aggregate values independent of the media, with some small variation (Figure 5). For CHO-DG44 (clone 1), 4Cell® SmartCHO supported low aggregation levels (Figure 5).

Figure 5: Product Aggregation in CHO Cells Cultured in 4Cell® SmartCHO vs. Commercial Media.



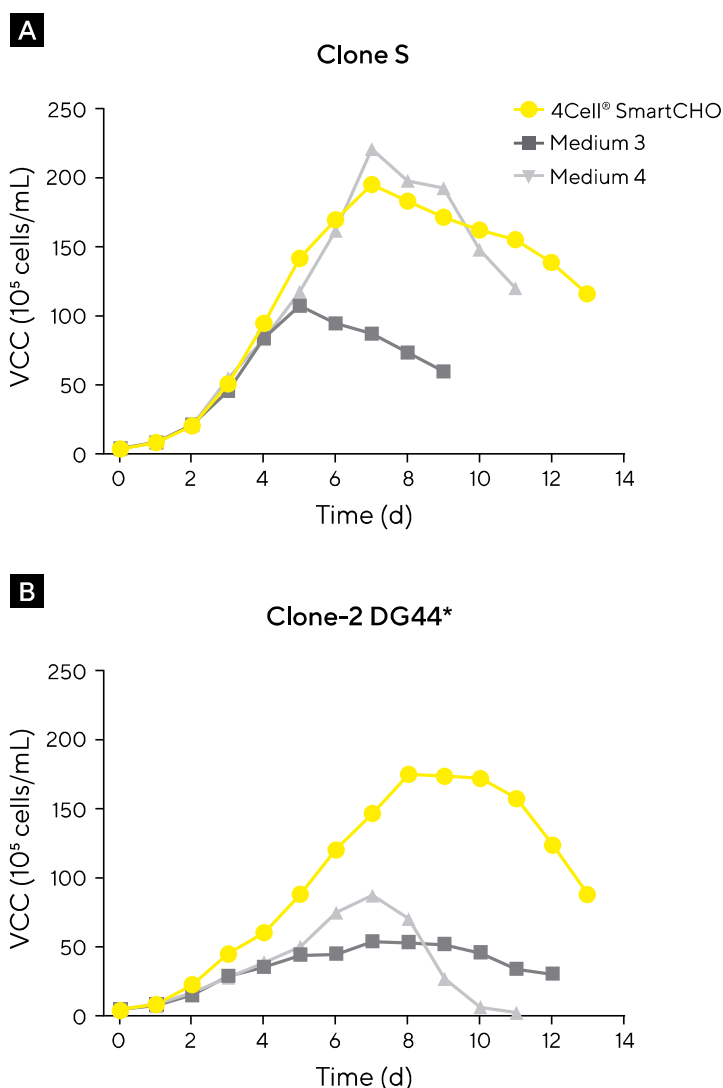
Note. Product aggregation determined by size exclusion chromatography.

Scalable Performance

Cell culture media performance must be consistent across scales. 4Cell® SmartCHO media shows robust and scalable growth performance when cultivations are carried out in a 5 L bioreactor (Figure 6).

4Cell® SmartCHO can support the production of high titers (Figure 7A) with high cell-specific productivity (Figure 7B) and lower aggregate formation (Figure 8) in large-scale cultures. This behavior was confirmed for different product classes (IgG1 and IgG4, data not shown).

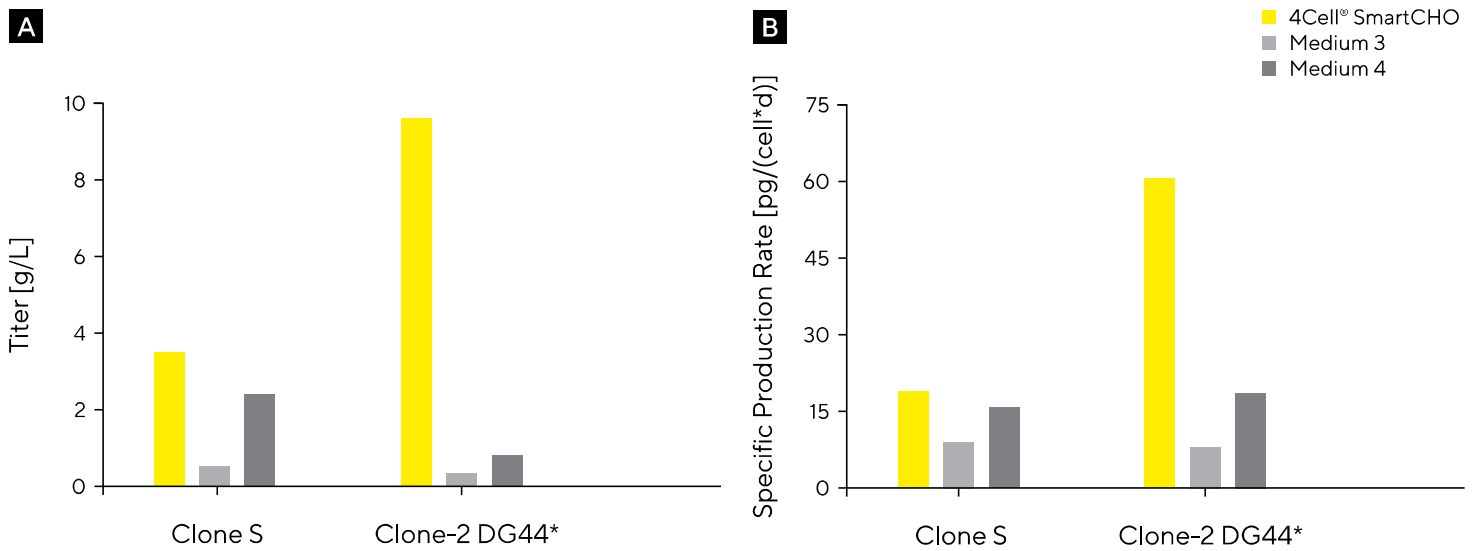
Figure 6: 4Cell® SmartCHO Media Supports Robust Growth of Two CHO Clones in 5 L Bioreactor Cultures.



Note. (A) CHO-S and (B) CHO-DG44 cells. Cells were adapted to the media for four weeks before testing. VCC = viable cell count.

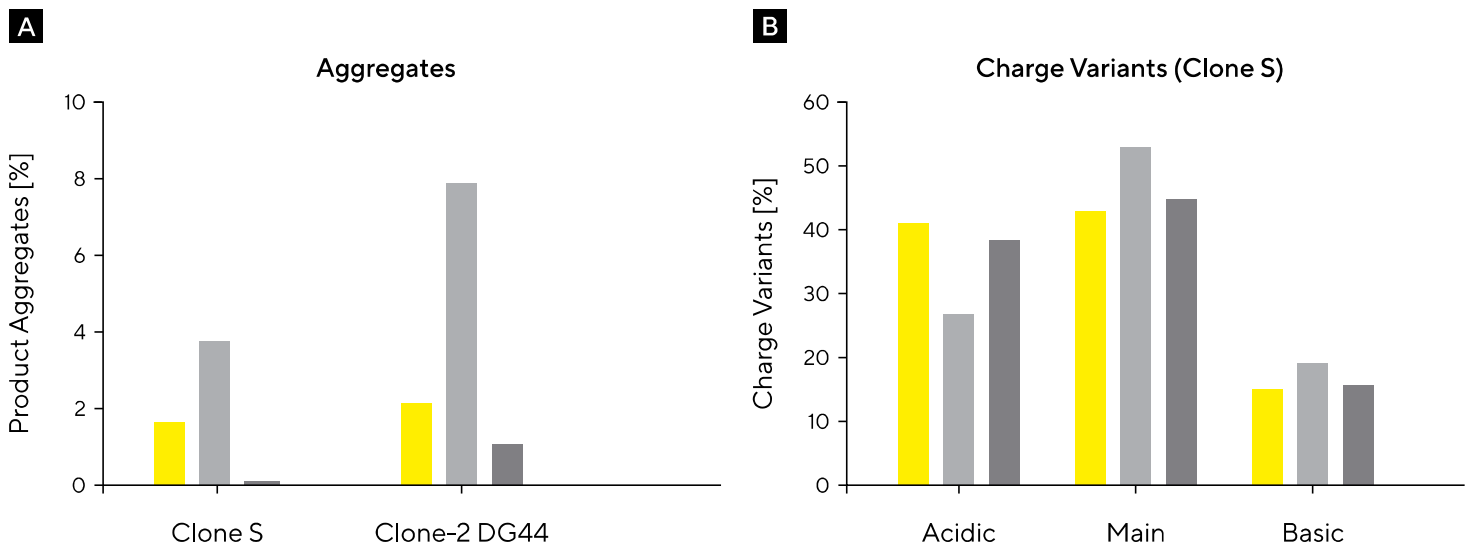
*Clone performance with Media 4 underestimated due to partial culture overfoaming.

Figure 7: Productivity of CHO-S and CHO-DG44 Cell Grown in 4Cell® SmartCHO vs. Commercial Media.



Note. (A) titer and (B) specific productivity.

Figure 8: Product Quality Attributes From CHO Cell Cultures Grown in 4Cell® SmartCHO vs. Commercial Media.



Note. (A) aggregates and (B) charge variants (CHO-S cells only).

4Cell® SmartCHO Supports the Cultivation of CHO-K1 Cells

In order to evaluate the performance of 4Cell® SmartCHO in different cell lines, a blind study was carried out by an external partner using a CHO-K1 cell line. This study demonstrated that the highest titer production was achieved using 4Cell® SmartCHO. This titer formation was even higher than regularly used customized media for that specific CHO-K1 clone (Table 1).

Table 1: Product Titers of CHO-K1 Cells Cultured in 4Cell® SmartCHO vs. Commercial Media, in Fed-batch Culture.

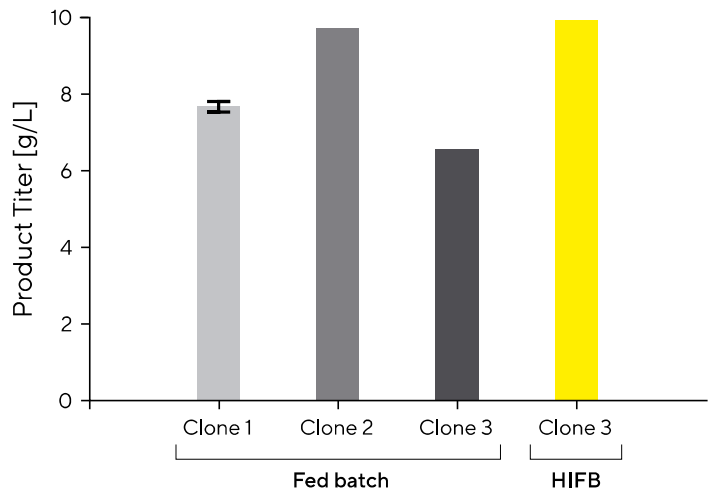
Medium	Titer
4Cell® SmartCHO	4.8 g/L
Medium A	2.1 g/L
Medium B	2.2 g/L
Medium C	3.8 g/L
Medium D	2.2 g/L

Note. Media Benchmarking performed with an external partner in a blind study.

4Cell® SmartCHO Supports the Transition to Perfusion and the Switch to SmartCHOpe

4Cell® SmartCHO also supports the application of protein intensification strategies. We tested three CHO-DG44 clones in fed-batch and high-inoculation fed-batch (HIFB) processes. The results showed that 4Cell® SmartCHO could support high titer in both standard and high inoculation fed-batch processes. This demonstrates the suitability of the media for intensified manufacturing strategies.

Figure 9: Productivity of 4Cell® SmartCHO Media Across Three CHO-DG44 Clones in Fed-Batch and High-Intensity Fed-Batch (HIFB) Processes.

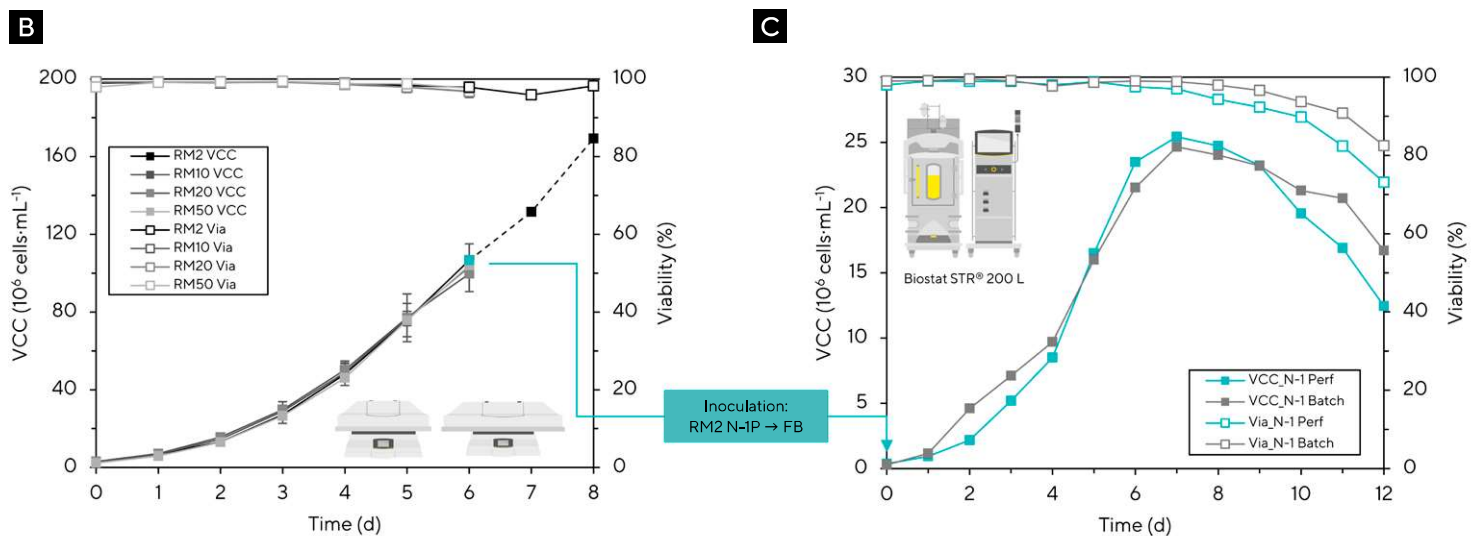
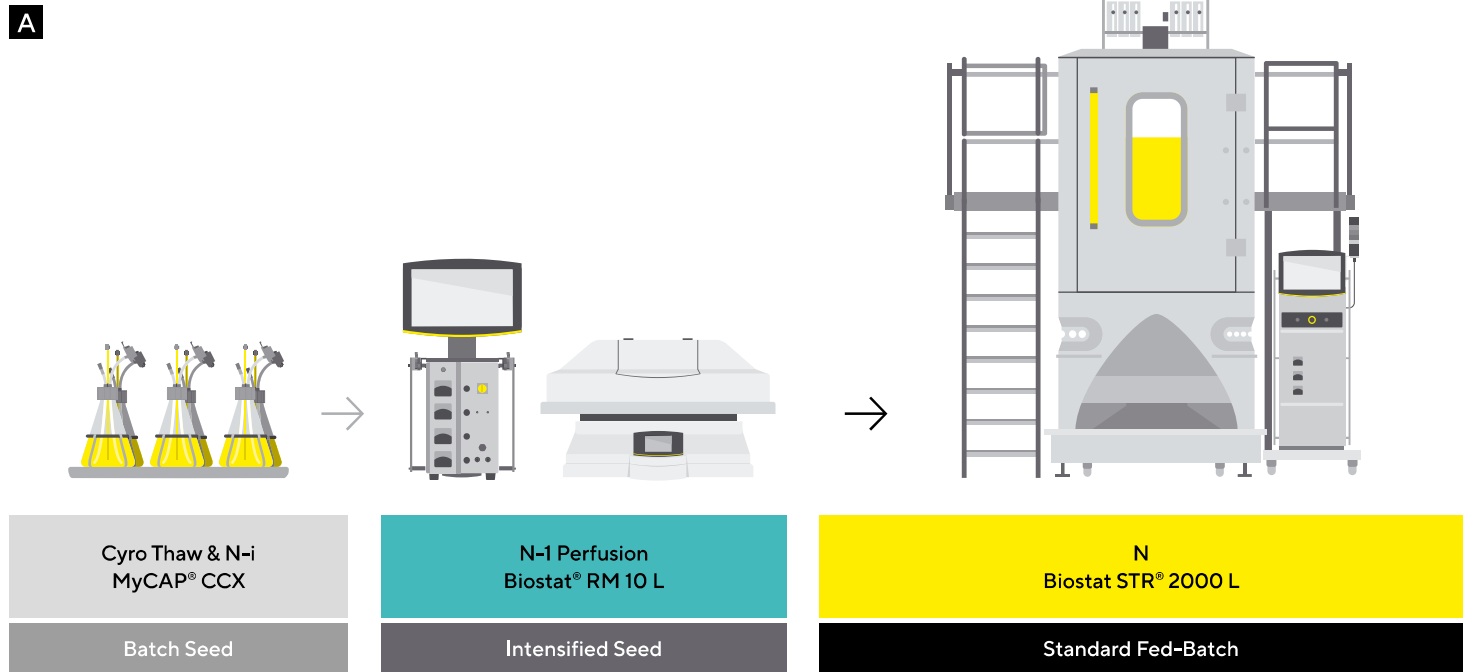


Note. Precision of titer measurements: ±10%.

4Cell® SmartCHOpe for Perfusion Applications

Our portfolio also includes a sister product of 4Cell® SmartCHO – 4Cell® SmartCHOpe – which provides a media solution for perfusion applications. 4Cell® SmartCHO and SmartCHOpe are easily interchangeable depending on the product, providing the flexibility to switch process formats without the cumbersome re-adaptation of cell lines.

Figure 10: Performance of 4Cell® SmartCHOpe in an N-1 Process.



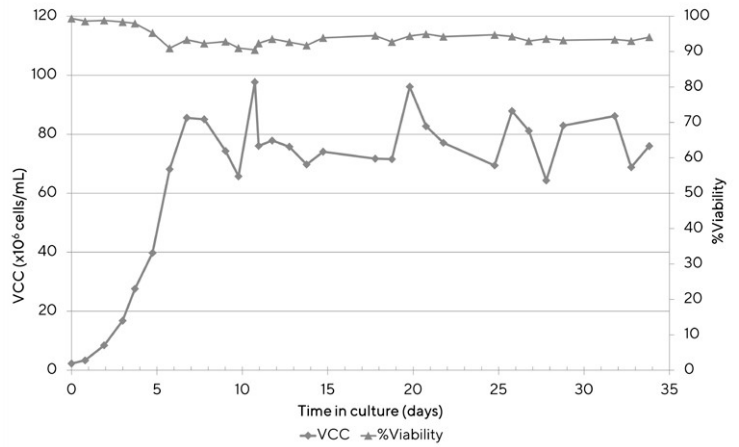
Note. (A) Seed train intensification by N-1 Perfusion, (B) Growth Performance of CHO cells cultured in 4Cell® SmartCHOpe across different seed train scenarios, (C) Growth Performance of CHO cells cultured in 4Cell® SmartCHOpe in a production bioreactor (Biostat® STR 200 L)

4Cell® SmartCHOpe can support process intensification by N-1 perfusion (Figure 10A). CHO cells were cultured in high-density perfusion cell cultures (100 million cells/mL) for 5-7 days (Figure 10B). This culture was then used to populate a 200 L bioreactor in standard fed-batch mode, showing comparable growth and viability to expansion in a standard seed train (Figure 10C).

4Cell® SmartCHOpe also supports continuous perfusion applications. Figure 11 shows data from a study in which a 2 L bioreactor with an ATF device was used to grow cells in 4Cell® SmartCHOpe media in continuous perfusion. Cells reached and maintained a high cell density, viability, and productivity, and importantly, the process was successfully run for 34 days.

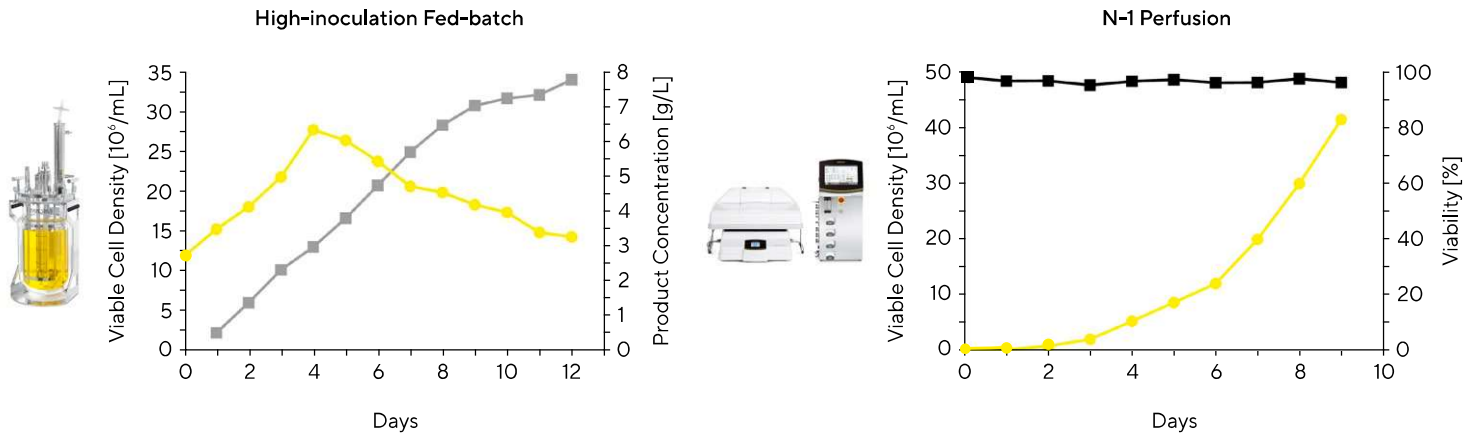
Using high inoculation fed-batch enabled by N-1 perfusion can help to increase productivity in the 10 g/L range depending on CHO clone (Figure 12).

Figure 11: 4Cell® SmartCHOpe Performance in Perfusion Processes.



Note. Viable cell density (VCD) and percentage viability were measured in a 2 L bioreactor with ATF for 34 days.

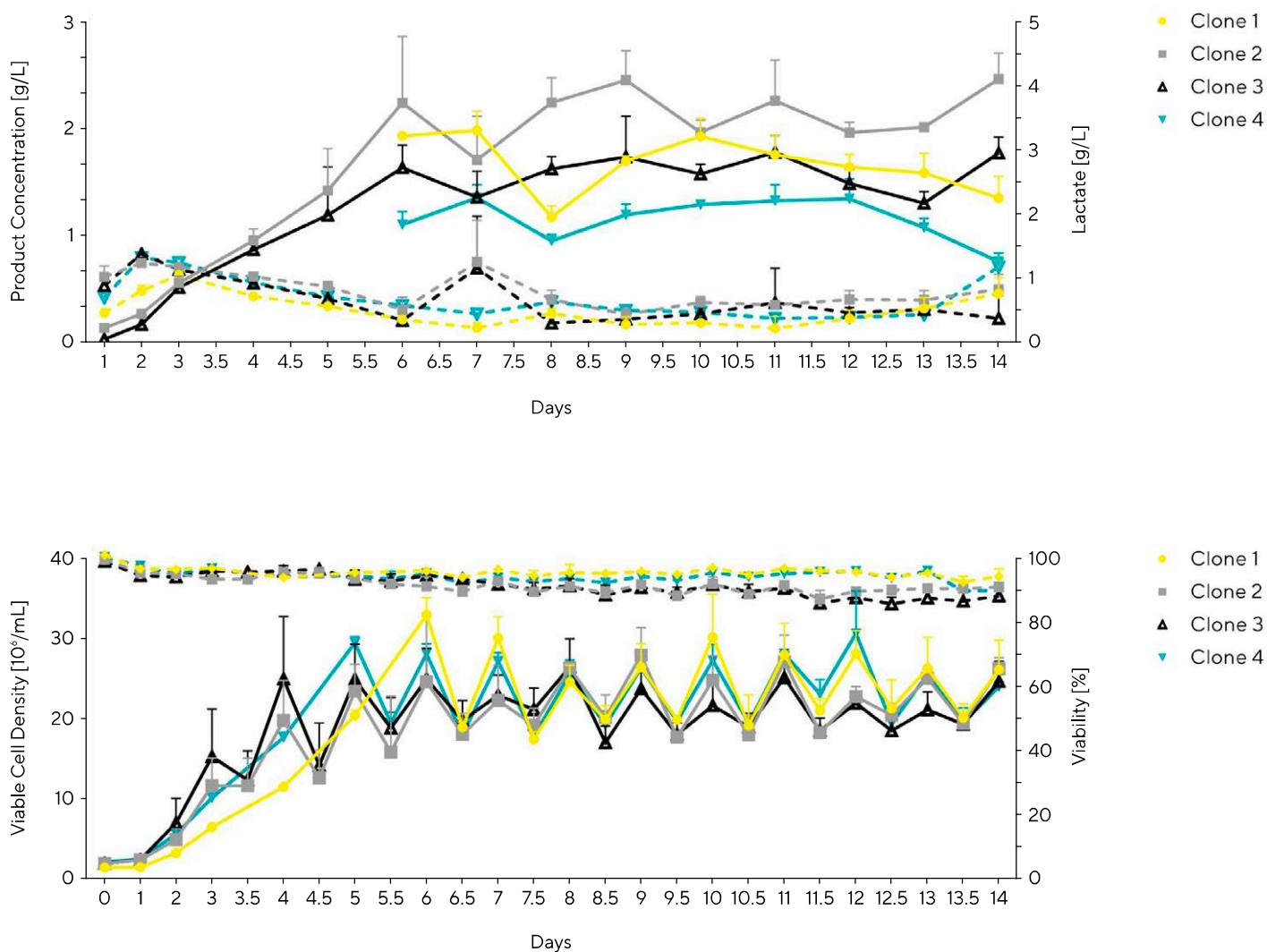
Figure 12: 4Cell® SmartCHOpe Performance in N-1 Perfusion Followed by High-Inoculation Fed-Batch.



Note. N-1 perfusion: cultivation was performed in the Biostat® RM System with 4Cell® SmartCHOpe at 1 L working volume. High-inoculation fed-batch cultivation was carried out in 5 L UniVessel® with 4Cell® SmartCHOpe as basal medium, and 1.3× 4Cell® SmartCHO feeds added to provide adequate nutrient supply throughout the whole process.

Finally, 4Cell® SmartCHOpE can be used with the Ambr® 15 perfusion mimic to support clone evaluation, which enables reliable clone selection for perfusion processes (Figure 13).

Figure 13: 4Cell® SmartCHOpE Performs Well in a Perfusion Mimic System.



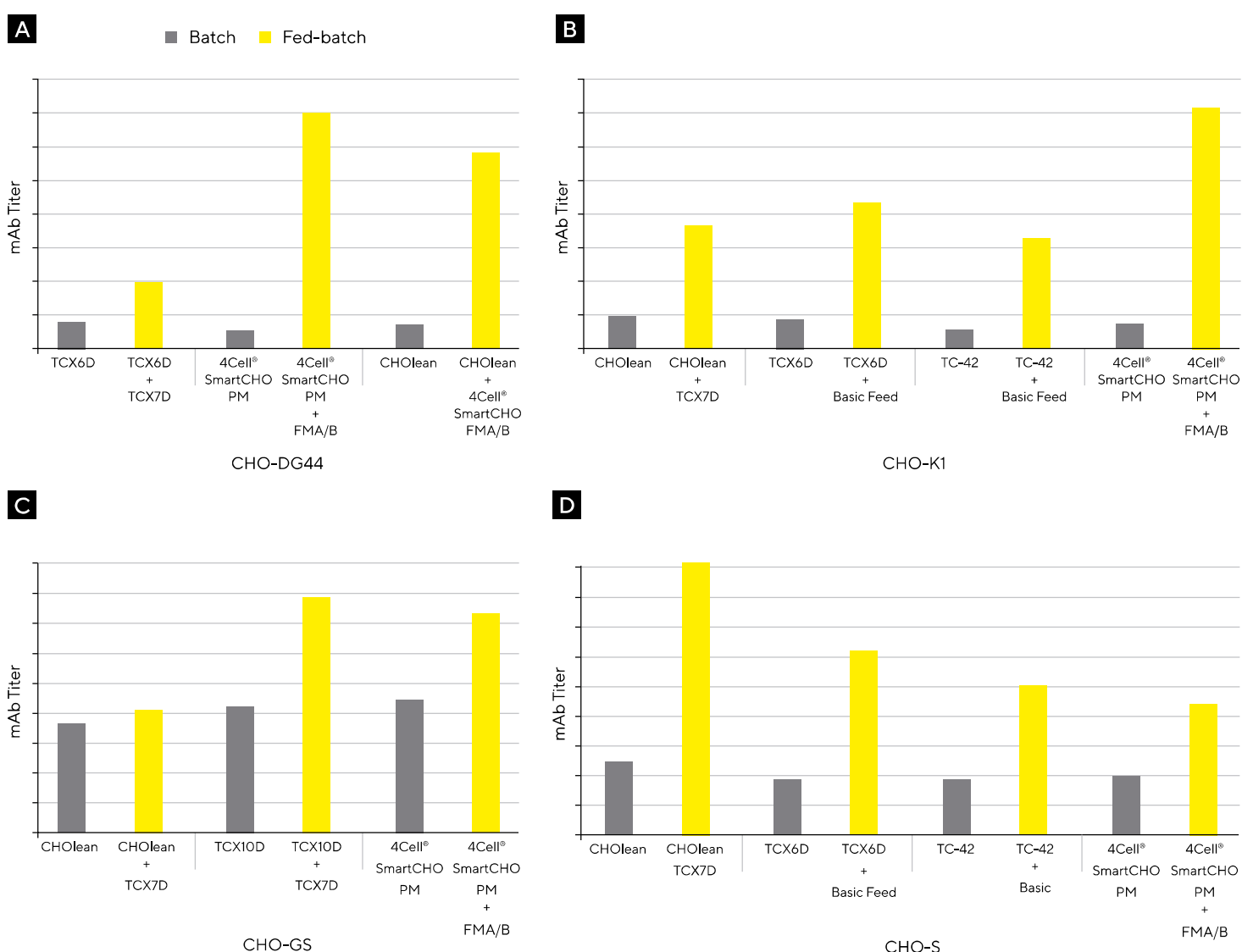
Note. Data from Cellca CHO-DG44 Cell Line Development Service. Four clones cultured in the Ambr® 15.

CHO Media and Feeds – Fed-Batch Comparisons

In addition to our advanced 4Cell® SmartCHO media, we also carry a broad portfolio suitable for a variety of cell lines, applications, and production strategies (Figure 1). We compared mAb production by four CHO cell lines cultured in Sartorius media in batch vs. fed-batch. These data show that the media and feeds produce different titers across the CHO cell lines, highlighting the importance of evaluating the performance of multiple media suitable to your process (Figure 14).

These data also highlight an important point: the titer achieved by a given media and feed combination in a batch process (usually adopted during screening) cannot be extrapolated to fed-batch titers (typically used during production). Therefore, it is important not to exclude media based on batch titers for fed-batch screening, as this can change dramatically when feeds are added later.

Figure 14: Performance of Various CHO media and Feed Combinations in Supporting Clones in Batch and Fed-Batch Processes.



Note. mAb titer produced in (A) CHO-DG44, (B) CHO-K1, (C) CHO-GS (D) CHO-S.

CHOlean – Ideal for Media Screening and Optimization

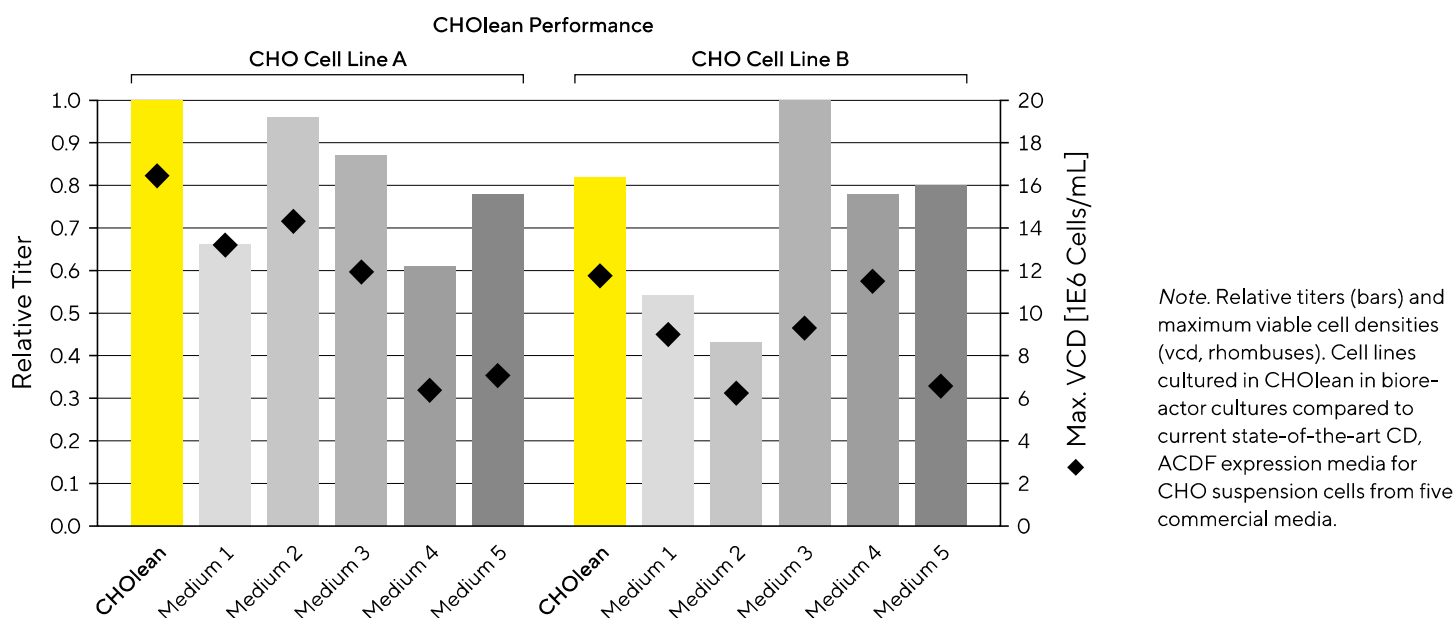
CHOlean is a lean formulation made with a minimal set of well-balanced components. It is our recommended starting point for media optimization studies, as it represents an off-the-shelf basal media that can be carefully supplemented to find the right formulation for each cell line. Its performance against commercial media is shown in Figure 15.

Transient Transfections with CHO-TF

CHO TF medium is the only medium in our portfolio that supports transient expression, as it is free from any components that might interfere with the transaction complex and prevent efficient transfection of cells (Figure 16).

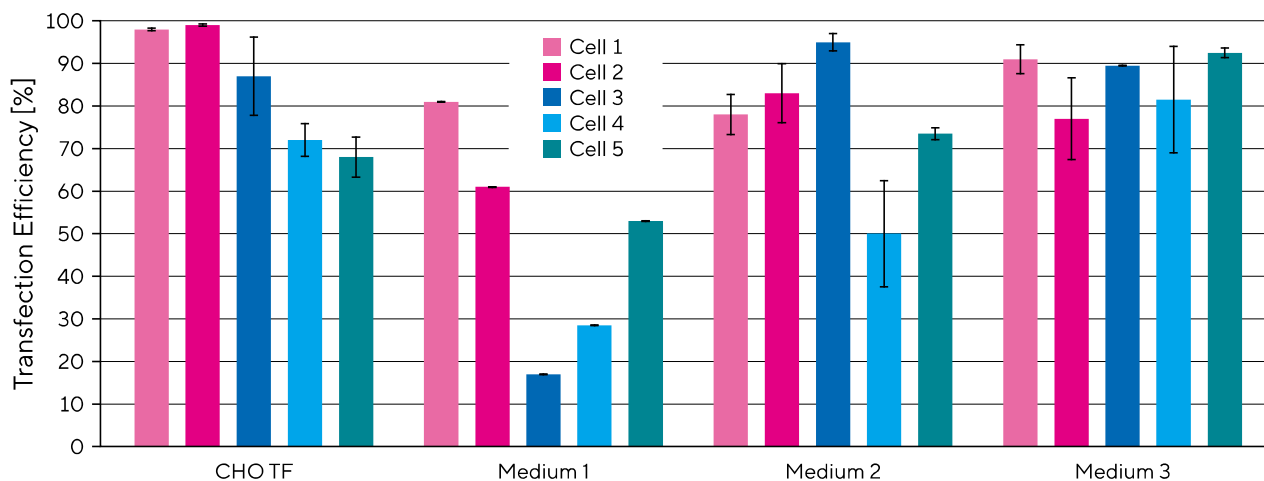
Transient expression is usually carried out in a batch expression rather than a fed-batch process; it is inefficient to prolong expression over several days, and typically short timelines are required.

Figure 15: Relative Titers and Maximum Viable Cell Densities of Two CHO Cell Lines in CHOlean vs. Commercial Media.



Note. Relative titers (bars) and maximum viable cell densities (vcd, rhombuses). Cell lines cultured in CHOlean in bioreactor cultures compared to current state-of-the-art CD, ACDF expression media for CHO suspension cells from five commercial media.

Figure 16: Transfection Efficiency Observed in Five CHO Cell Lines in CHO TF vs. Commercial Media.



Note. Transfection efficiency was measured as GFP expression. Commercial media were current state-of-the-art CD, ADCF expression media for CHO suspension cells. Fresh medium was used at the time of transfection.

Technical Specifications

Medium	Surfactant	Phenol Red	L-Glutamine	Hypoxanthine/ Thymidine	Growth Factors	Glucose	Amino Acids	Vitamins
SmartCHO® (SAM PM)	■	–	–	–	–	■	■	■
SmartCHO® FMB	–	–	–	–	–	–	■	–
SmartCHO® FMA	■	–	–	–	–	■	■	■
TCXD6	■	–	–	■	■	■	■	■
TC-42 w/IGF	■	–	–	–	■	■	■	■
TC-42 w/rInsulin	■	–	–	–	–	■	■	■
TC-42 w/o GF	■	–	–	–	–	■	■	■
TCX10D	■	–	–	■	–	■	■	■
CHOlean	■	–	–	–	–	■	■	■
CHO TF	■	–	–	■	–	■	■	■
TCX7D feed	■	–	–	■	–	60 g/L	■	■
Basic Feed	■	–	–	■	–	20 g/L	■	■

Ordering Information

CHO Media Sample Kits

Product Name	Medium	Format Quantity Package	Order Number
4Cell® SmartCHO Media System	Stock & Adaptation Medium (SAM)	Liquid 1 L Bottle	CFP3FB0200
	Production Medium (PM)	Liquid 2 × 1 L Bottles	
	Feed Medium A (FMA)	Liquid 1 L Bottle	
	Feed Medium B (FMB)	Liquid 125 mL Bottle	
4Cell® SmartCHOpe Media System	Stock & Adaptation Medium (SAM)	Powder 1 × 10 L Container	CQP3FC0100
	Production Medium (PM)	Powder 1 × 50 L Container	
	Feed Medium A (FMA)	Powder 2 × 3 L Container	
	Feed Medium B (FMB)	Powder 1 × 2 L Container	
CHO-GS Media Kit	TCX10D w/o DS	Liquid 1 L Bottle	CFP3FF0200
	TCX7D Feed	Liquid 1 L Bottle	
CHO Media Kit	TCX6D	Liquid 1 L Bottle	CFP3FF0201
	TC-42 w/o IGF	Liquid 1 L Bottle	
	CHOlean	Liquid 1 L Bottle	
	Basic Feed	Liquid 1 L Bottle	

CHO Media Portfolio

Medium	Description	Format Quantity Package	Order Number
CHO TF	Ideal medium for working with transient CHO cells	Liquid 1 L Bottle	886-0001
		Liquid 1 L Bottle (for SILAC*)	888-0001
		Powder 10 L Container	886-0010DPM
		Powder 50 L Container	886-0050DPM
CHOlean	<ul style="list-style-type: none"> ▪ Lean and robust growth medium for a variety of applications ▪ Lean formulation with minimal components 	Liquid 1 L Bottle	1140-0001
		Liquid 1 L Bottle (for SILAC*)	1141-0001
		Powder 10 L Container	1140-0010DPM
		Powder 50 L Container	1140-0050DPM
		Powder 100 L Container	1140-0100DPM
TC-42	Workhorse formulation especially designed for CHO-DHFR ⁻ cell lines	Liquid 1 L Bottle (w/ IGF)	510-0001
		Liquid 1 L Bottle (w/ Insulin)	509-0001
		Liquid 1 L Bottle	511-0001
		Liquid 1 L Bottle (for SILAC*)	512-0001
		Powder 10 L Container	511-0010DPM
		Powder 50 L Container	511-0050DPM
TCX10D	For long-term high-performance growth with CHO-GS cells	Liquid 1 L Bottle (w/ Dextran Sulfate)	1100-0001
		Liquid 1 L Bottle (w/o Dextran Sulfate)	1150-0001
		Powder 10 L Container (w/o Dextran Sulfate)	1150-0010DPM
		Powder 50 L Container (w/o Dextran Sulfate)	1150-0050DPM
		Powder 100 L Container (w/o Dextran Sulfate)	1150-0100DPM
		Powder 200 L Container (w/o Dextran Sulfate)	1150-0200DPM
		Powder 500 L Container (w/o Dextran Sulfate)	1150-0500DPM
TCX6D	One high-performing medium for any CHO Clone with growth factor LONG [®] R3IGF-I	Liquid 1 L Bottle	1070-0001
		Powder 10 L Container	1070-0010DPM
		Powder 50 L Container	1070-0050DPM
		Powder 100 L Container	1070-0200DPM
		Powder 200 L Container	1070-0250DPM
		Powder 500 L Container	1070-0500DPM
TCX7D Feed Supplement	With 60 g/L D-glucose; esp. for CHO GS cell lines.	Liquid 1 L Bottle	1080-0001
		Powder 2 L Container	1080-0002DPM
		Powder 5 L Container	1080-0005DPM
		Powder 10 L Container	1080-0010DPM
		Powder 25 L Container	1080-0025DPM
		Powder 50 L Container	1080-0050DPM
		Powder 100 L Container	1080-00100DPM
Basic Feed	With 20 g/L D-glucose. Also suitable for hybridoma.	Liquid 1 L Bottle	1092-0001
		Liquid 10 L Bag	1092-0010BAG
		Liquid 20 L Bag	1092-0020BAG
		Liquid 50 L Bag	1092-0050BAG
		Powder 5 L Container	1092-0005DPM
		Powder 10 L Container	1092-0010DPM
		Powder 50 L Container	1092-0050DPM

*SILAC means w/o lysine and arginine ideal for expression of labeled proteins

Medium	Description	Format Quantity Package	Order Number
4Cell® SmartCHO SAM	Allows the cultivation or media adaptation of CHO cells	Liquid 2 × 1 L Bottles	CFP3FB1106
		Powder 10 L Container	CQP3FB1146
		Powder 25 L Container	CQP3FB1147
		Powder 250 L Container	CQP3FB1148
4Cell® SmartCHO PM	Non-selective production medium for batch or for fed batch culture. It can be used for effective cultivation of any CHO cell lines, and helps produce high yield of the active protein.	Liquid 2 × 1 L Bottles	CFP3FB2107
		Powder 9 L Container	CQP3FB2149
		Powder 20 L Container	CQP3FB2150
		Powder 200 L Container	CQP3FB2151
4Cell® SmartCHO FMA	Ideal companion feeds designed to be used together with 4Cell® SmartCHO Production Medium	Liquid 2 × 1 L Bottles	CFP3FB3108
		Powder 3 L Container	CQP3FB3152
		Powder 30 L Container	CQP3FB3153
4Cell® SmartCHO FMB	Ideal companion feeds designed to be used together with 4Cell® SmartCHO Production Medium	Liquid 2 × 125 mL Bottles	CFP3FB4109
		Liquid 2 × 500 mL Bottles	CFP3FB4110
		Powder 2 L Container	CQP3FB4154
		Powder 4 L Container	CQP3FB4155
		Powder 45 L Container	CQP3FB4156
4Cell® SmartCHOpe SAM	Allows the cultivation or media adaptation of CHO cells	Powder 10 L Container	CQP3FC1101
		Powder 25 L Container	CQP3FC1102
		Powder 250 L Container	CQP3FC1103
4Cell® SmartCHOpe PM	Non-selective production medium for perfusion culture.	Powder 20 L Container	CQP3FC2102
		Powder 50 L Container	CQP3FC2101
		Powder 200 L Container	CQP3FC2103
4Cell® SmartCHOpe FMA	Ideal companion feeds designed to be used together with 4Cell® SmartCHOpe Production Medium	Powder 3 L Container	CQP3FC3103
		Powder 30 L Container	CQP3FC3102
		Powder 120 L Container	CQP3FC3104
4Cell® SmartCHOpe FMB	Ideal companion feeds designed to be used together with 4Cell® SmartCHOpe Production Medium	Powder 2 L Container	CQP3FC4104
		Powder 4.5 L Container	CQP3FC4101
		Powder 45 L Container	CQP3FC4102
		Powder 180 L Container	CQP3FC4103

Germany

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

Sartorius Xell GmbH
Waldweg 21
33758 Schloss Holte-Stukenbrock
Phone +49 521 96989 200

USA

Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

 **For more information, visit**
www.sartorius.com