

A Guide to Serum-Free Cell Culture



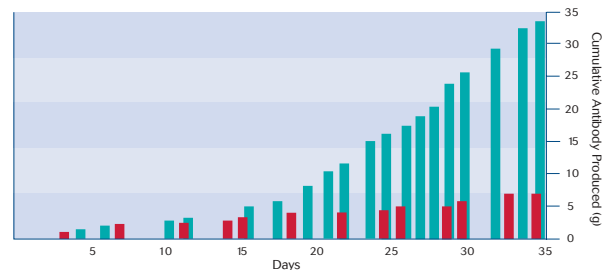
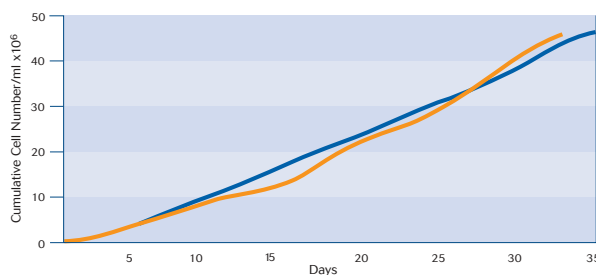
Boost productivity, meet regulatory requirements, and simplify purification.

Researchers who “think downstream” choose GIBCO® products for serum-free cell culture.

If you’re working with cells that may ultimately be used in genomic, proteomic, biopharmaceutical, therapeutic, or diagnostic applications, your choice of culture media becomes more critical than ever.

GIBCO® media meet pharmaceutical standards for quality, and are the most approved in the biopharmaceutical industry because they:

- Minimize variables
- Maximize yield
- Simplify purification
- Meet regulatory requirements
- Optimize results



Cumulative MAb Production and Number of Cells. Cells were cultured in Hybridoma-SFM (orange bars) or D-MEM with FBS (blue bars) (10% until day 10, 7.5% until day 13, and 5% from day 13). Cell number and MAb production were monitored. The culture with Hybridoma-SFM produced significantly more MAbs even though the number of cells was similar to serum-supplemented medium.

Optimize Serum-Free Cell Culture with GIBCO® Products

- Choose from chemically-defined, protein-free, and serum-free formulations
- Minimize risk with animal-origin-free media and reagents
- Save time with pre-adapted cells
- Use liquid or Advanced Granulation Technology™ (AGT™) formats that are scalable to large volume cultures

Check the chart in the center of this guide to see which GIBCO® medium is right for your particular cell line. Custom products and packaging are also available.

SEM

Serum-Free Media

GIBCO® Serum-Free Media do not require supplementation with serum, but may contain discrete proteins or bulk protein fractions.

PFM

Protein-Free Media

GIBCO® Protein-Free Media contain no proteins, but may contain plant or yeast hydrolysates. Many are animal-origin-free.

CDM

Chemically-Defined Media

GIBCO® Chemically-Defined Media contain no proteins, hydrolysates, or components of unknown composition. These media are animal-origin-free and all components have a known chemical structure.

- Completely defined system eliminates variability
- Consistent performance improves reproducibility
- Decrease possibility of contamination by adventitious agents
- Save time with simplified purification and downstream processing

Minimize Risk with Animal-Origin-Free Media and Reagents

Materials of animal-origin have the potential to introduce adventitious agents into a cell culture system.

At Invitrogen we have developed a wide range of cell culture products that are completely free of animal-origin materials by taking multiple steps to ensure the quality, safety, consistency, and regulatory compliance of our products:

- Sourcing non-animal-origin raw materials whenever possible
- Substituting bioactive synthetic chemicals for their native animal-derived counterparts
- Ensuring solid documentation that details source, origin, and manufacturing process for all raw materials
- Using non-protein compounds instead of insulin and transferrin in protein-free and chemically-defined media

GIBCO® serum-free products that are animal-origin-free are noted on the chart in the center of this guide. Additional GIBCO® animal-origin-free products include rProtease™, a novel, microbially-produced alternative to animal trypsin, and 250X Cholesterol Lipid Concentrate, among others.



Speed Your Progress with Outsourced Support From Cell Culture Experts

As you look to outsource your development activities, turn to us. The multifaceted scientific and engineering expertise that brings you the world's best cell culture materials is now available to provide you with outsourced support.

We can configure virtually any of our capabilities to suit your needs, and help integrate and optimize your process through all phases of process development and biomanufacturing.

Custom Media Production and Packaging

When you need a unique formulation or special packaging, our Custom Product Services team can modify GIBCO® catalog media formulations and packaging to meet your particular requirements.

The Custom Product Services team can also assess feasibility and provide options for formulation design, testing, and packaging for your proprietary formulations.

We can produce volumes as small as a few liters to > 30,000 liters, or > 100,000 liters in dry format. In addition, we offer large media bag packaging options up to 500 liters.

Media Development

In a comprehensive approach, our R&D scientists can create the optimal nutritional environment, developing media formulations specifically suited to your working cell lines. We can formulate nutrient supplements to your specifications for bioreactor feed strategies.

GIBCO® animal-origin-free products do not contain material directly derived from animal tissues, cells, or body fluids of higher eukaryotic organisms, such as mammals (including humans), fish, birds, insects, etc. The term "animal-origin" does not pertain to lower eukaryotic organisms such as the higher plants, fungi, protozoa, and algae, nor does it include prokaryotic organisms such as bacteria or blue-green algae.

Media Optimization

Media optimized to meet the nutritional requirements of your cell line can help ensure that your system operates at maximum efficiency and consistency.

Through basal media design, fed-batch supplement design, and the establishment of feeding regimens, we can provide the best nutrient media delivery scheme for your recombinant cell line, optimizing a GIBCO® medium, one in the public domain, or your own formulation.

Cell Adaptation

We can help you save time by adapting your cells to perform in GIBCO® serum-free, protein-free, or chemically-defined media.

Our goal is to establish your cell line in a stable cell culture environment that is transferable and scalable.

Media Analytical Services

Characterizing intermediates and their impact on final products is a significant part of preclinical and process development efforts.















You can use our analytical expertise to get the most from your cell growth system. We can help determine potential improvements to your formulation by examining spent media samples for changes in amino acid and water-soluble vitamin levels. Our capabilities also include analysis of glucose/lactate, and selected lipids and cations.

Global Manufacturing

Invitrogen cell culture manufacturing facilities in Grand Island, New York, USA; Paisley, Scotland, UK; and Auckland, New Zealand are ISO-9001 certified and compliant with guidelines established by the FDA's Quality System Regulation (QSR/cGMP). In addition, we have a Drug Master File for several products.



GIBCO™ Serum-Free Media and Pre-Adapted Cells

Product	Optimized For	Applications	Size	Catalog No.
Hybridoma Culture				
 CD Hybridoma Medium†	Human, mouse, rat hybridomas, myelomas. NS0, NS-1, and other steroid-dependent cells when used with 250X Cholesterol Lipid Concentrate	Growth and MAb production. Can be used to express other proteins in engineered myeloma cell lines.	500 ml	11279-015
			1,000 ml	11279-023
 CD Hybridoma AGT™† Dry granular format of CD Hybridoma Medium.			1 X 1 L	12372-025
			1 X 10 L	12372-017
Hybridoma-SFM† Low-protein 20 µg/ml.	Human, mouse, rat hybridomas, myelomas	Growth and MAb production. Can be used to express other proteins in engineered myeloma cell lines.	500 ml	12045-084
			1,000 ml	12045-076
 PFHM-II Protein-free Hybridoma Medium.	Human, mouse, rat hybridomas, myelomas	Growth and MAb production. Can be used to express other proteins in engineered myeloma cell lines.	1,000 ml	12040-077
Chinese Hamster Ovary (CHO) Cell Culture				
 CD CHO Medium†	Suspension CHO cells (including CHO-S cells)	Growth and production of recombinant proteins.	500 ml	10743-011
			1,000 ml	10743-029
 CD CHO Medium AGT™† Dry granular format of CD CHO Medium.			1 X 1 L	12490-017
			1 X 10 L	12490-025
CD CHO-A Medium	Adherent CHO cells		500 ml	097-0182DJ
			1,000 ml	097-0182DK
CHO-S-SFM II† Available without Hypoxanthine and Thymidine; cat. no. 31033-020.	Suspension CHO cells (including CHO-S cells)	Growth and production of recombinant proteins in suspension culture.	500 ml	12052-114
			1,000 ml	12052-098
 CHO III PFM†	Suspension CHO cells (including CHO-S cells)	Growth and production of recombinant proteins in suspension culture.	500 ml	096-0334DJ
			1,000 ml	096-0334SA
 CHO III-A-PFM	Adherent CHO cells	Growth and production of recombinant proteins in adherent culture.	1,000 ml	097-0147DK
CHO-S Cells, Adapted to CD CHO Medium, CHO-S-SFM II and CHO III PFM			1.5 ml	11619-012
Human Embryonic Kidney (293) Cell Culture				
 CD 293 Medium†	Suspension 293 cells (including 293-F, 293-H)	Growth and recombinant protein or adenovirus production in suspension culture.	1,000 ml	11913-019
			1 X 1 L	12529-020
 CD 293 AGT™† Dry granular format of CD 293 Medium.			1 X 10 L	12529-012
 293 SFM II† Low-protein < 10µg/ml.	Suspension 293 cells and HeLa S3 cells	Growth and recombinant protein or adenovirus production in suspension culture.	500 ml	11686-011
			1,000 ml	11686-029
 FreeStyle™ 293 Expression Medium	Suspension 293-F cells	Growth and transfection in suspension culture.	1,000 ml	12338-018
			6 X 1,000 ml	12338-026
293-F Cells, Adapted to CD 293 Medium and 293 SFM II			1.5 ml	11625-019
293-H Cells, Adapted to CD 293 Medium and 293 SFM II			1.5 ml	11631-017
FreeStyle™ 293-F Cells, Adapted to FreeStyle™ 293 Expression Medium			1 X 10 ⁷ cells	R790-07
Mammalian Cell Culture for Virus Production				
 VP-SFM† Low-protein < 10µg/ml.	Suspension BHK-21 cells Adherent VERO, COS-7L, MDCK, HEp-2	For culture of kidney epithelial and related cells used in virus production.	1,000 ml	11681-020
			1 X 1 L	12559-027
 VP-SFM AGT™† Dry granular format of VP-SFM			1 X 10 L	12559-019
 OptiPro™ SFM† Low-protein < 10µg/ml.	Adherent MDCK, VERO, PK-15, MDBK, BHK-21	For culture of kidney epithelial and related cells used in virus production.	1,000 ml	12309-019
COS-7L Cells, Adapted to VP-SFM			1.5 ml	11622-016
Insect Cell Culture				
Drosophila-SFM	Suspension <i>Drosophila melanogaster</i> cells (D.Mel-2, Schneider S2 cells)	Growth and maintenance medium for adherent or suspension culture.	500 ml	10797-017
			1,000 ml	10797-025
Express Five® SFM	Suspension BTI-TN-5B1-4 insect cells	Growth and maintenance of cells used for the baculovirus expression vector system (BEVS) for adherent or suspension culture. Large-scale production of recombinant protein expressed by BEVS.	1,000 ml	10486-025
Sf-900 II SFM†	Suspension Sf9, Sf21, (<i>Spodoptera frugiperda</i>), TN368 cells (<i>Trichoplusia ni</i>)	Growth and maintenance of cells used for BEVS for adherent or suspension culture. Large-scale production of recombinant protein expressed by BEVS.	500 ml	10902-096
			1,000 ml	10902-088
			10 L	10902-070
D.Mel-2 Cells, Adapted to Drosophila SFM			1.5 ml	10831-014
High Five™ Cells, Adapted to Express Five® SFM			3 X 10 ⁶ cells/ml	B855-02
Sf9 Cells, Adapted to Sf-900 II SFM			1.5 ml	11496-015
Sf21 Cells, Adapted to Sf-900 II SFM			1.5 ml	11497-013

Product	Optimized For	Applications	Size	Catalog No.
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Neuronal Cell Culture

Neurobasal™ Medium	Fetal neurons	Basal medium lacking excitatory amino acids used or in conjunction with supplements to make a complete serum-free medium. Long-term growth of neurons.	500 ml	21103-049	
Neurobasal™ Medium without Phenol Red			500 ml	12348-017	
Neurobasal™-A Medium			Adult and postnatal neurons (> 1 week old)	500 ml	10888-022
Neurobasal™-A Medium without Phenol Red				500 ml	12349-015
with B-27 Serum-Free Supplement	Primary embryonic hippocampal neurons; primary neurons from striatum, substantia nigra, septum	Growth and maintenance. Minimizes glial cell proliferation. B-27 minus AO to study free-radical damage, apoptosis. (B-27 minus AO is formulated without any cortical antioxidants).	10 ml	17504-044	
with B-27 Supplement Minus AO			10 ml	10889-038	
with B-27 Supplement Minus Vitamin A (Retinoic Acid)			10 ml	0980153A	
with N-2 Supplement	Primary embryonic hippocampal neurons, tumor cell lines of neuronal origin (PC12, B104, N1E-115 and NS20)	Maintenance of primary neurons (low protein, < 125 µg/ml). Growth and maintenance of neuronal tumor cell lines.	5 ml	17502-048	
with G-5 Supplement	Primary glial cells, tumor cell lines of glial origin (U-251, MGsp, C62BD, RN-22), astrocytes, microglia, oligodendrocytes	Growth and maintenance of primary and serial tumor glial cells.	1 ml	17503-012	

Blood and Bone Marrow Cell Culture

AIM V® Medium, liquid† Research Grade, with HSA.	Lymphocytes, macrophages, monocytes, lymphoid cell lines	<i>Ex vivo</i> activation of cytotoxic lymphocytes with IL-2 supplementation.	500 ml	12055-091
AIM V® Medium, liquid Therapeutic Grade.			1,000 ml	12055-083
Macrophage-SFM	Macrophages, monocytes	Growth of tumor infiltration lymphocytes (TIL cells), cytotoxic T-cells, and monocytes.	1,000 ml 10 L	087-0112DK 087-0112BK
StemPro®-34 SFM Supplied with hematopoietic StemPro®-Nutrient Supplement.	Human hematopoietic progenitor cells (CD34+) from bone marrow, peripheral blood, or neonatal cord blood	Growth and maintenance (addition of GM-CSF may be necessary). Demonstration of macrophage phagocytosis. Activation of cells to kill tumor cells with γ interferon or lipopolysaccharide supplementation.	500 ml	12065-074
		Growth and maintenance of human hematopoietic progenitor cells (addition of factors necessary). Study synergistic/individual effects of growth.	500 ml	10639-011

Other Mammalian Cell Culture

Human Endothelial-SFM†	Primary and secondary human umbilical venous, microvascular, and arterial endothelial cells	Growth and maintenance to study cell-cell interactions, injury analysis, atherosclerosis, signal transduction, cytokine production, and cell matrix interaction. Requires supplementation with bFGF, EGF, and fibronectin. Sold separately.	500 ml	11111-044
Hepatozyme-SFM	Primary human, monkey, and rat hepatocytes	Maintenance of hepatocytes (cytochrome P450 induction maintained > 9 days).	500 ml	17705-021
Keratinocyte-SFM (with EGF, BPE)†	Human epidermal keratinocytes and cervical epithelial cells (will not support fibroblast or melanocyte cells)	Growth and maintenance for dermal substitutes, gene therapy, and <i>in vitro</i> toxicology.	500 ml	17005-042
Keratinocyte-SFM (without CACI ₂)†			Without bovine pituitary.	500 ml
Defined Keratinocyte-SFM†	Human epidermal keratinocytes and cervical epithelial cells (will not support fibroblast or melanocyte cells)	Low-protein (< 25 µg/ml) without BPE medium for cultivation of keratinocytes. Can be used for cervical epithelial cells for studies involving the human papilloma virus.	500 ml	10744-019
KnockOut™ D-MEM	Murine and human embryonic stem (ES) cells	Growth and maintenance of undifferentiated ES cells for production of transgenic mice. Growth and maintenance of both human and murine ES cells used in differentiation studies.	500 ml	10829-018
KnockOut™ Serum Replacement			500 ml	10828-028
Primary Human Keratinocytes, Adapted to Defined Keratinocyte-SFM			1 ml	12332-011

Chemically-Defined Media

Protein-Free Media

Serum-Free Media

Pre-Adapted Cells



Animal-Origin-Free Product

How to Convert from Serum-Supplemented to Serum-Free Media

When you're ready to switch to serum-free cell culture, we're ready to help. Here are some important tips on the process from our Cell Culture Technical Service Specialists.

Sequential Adaptation

Sequential adaptation is Invitrogen's preferred method for adapting cells to serum-free media (SFM), with a typical conversion being:

Passage 1
75% serum-supplemented medium: 25% SFM
Passage 2
50% serum-supplemented medium: 50% SFM
Passage 3
25% serum-supplemented medium: 75% SFM
Passage 4
100% SFM

Because the change from 75% to 100% SFM may be too stressful for your cells, you may need to carry the cells for 2–3 passages in a 10% serum-supplemented medium: 90% SFM mixture. Most cell lines can be considered fully adapted after 3 passages in 100% SFM. Occasionally you may have trouble getting your cells past a certain step even before going 100% SFM. If this happens, go back and passage the cells 2–3 times in the previous ratio of serum-supplemented media to serum-free media.

Adaptation with Conditioned Medium

An alternate method for adaptation to SFM involves the use of “conditioned medium.” This is medium the cells have been growing in for one full passage. If you choose this method, you can facilitate adaptation as follows:

Passage 1
100% serum-supplemented medium
Passage 2
50% medium from passage 1: 50% SFM
Passage 3
50% medium from passage 2: 50% SFM
Passage 4
50% medium from passage 3: 50% SFM
Passage 5
100% SFM

Whichever adaptation method you choose, we strongly recommend that you always take these precautions:

- Make a frozen stock of the cells in the serum-supplemented media prior to adaptation.
- Keep a culture going of the cells in each prior condition when starting the next level of adaptation as a fall-back if the cells do not survive in the next passage.

If you have questions that these tips don't address, call 1-800-955-6288 or e-mail tech_service@invitrogen.com

Points to Consider in Serum-Free Culture

Overall, cells in serum-free culture are more sensitive to extremes of pH, temperature, osmolality, mechanical forces, and enzyme treatment.

Antibiotics

It is best not to use antibiotics in serum-free media. If you do, we recommend that you use 5- to 10-fold less than you would in a serum-supplemented medium. This is because serum proteins tend to bind a certain amount of the antibiotic added; without these serum proteins the level of antibiotic may be toxic to certain cells.

Higher Density

Cells must be in the mid-logarithmic phase of growth with viability > 90% prior to adaptation. Sequential adaptation may be necessary.

Seeding cultures at a higher density than normal at each passage during SFM adaptation may help the process. Because some percentage of cells may not survive in the new culture environment, having more cells present will increase the number of viable cells to further passage.

Clumping

Cell clumping often occurs during adaptation to SFM. We recommend that you gently triturate the clumps to break them up when passaging cells.

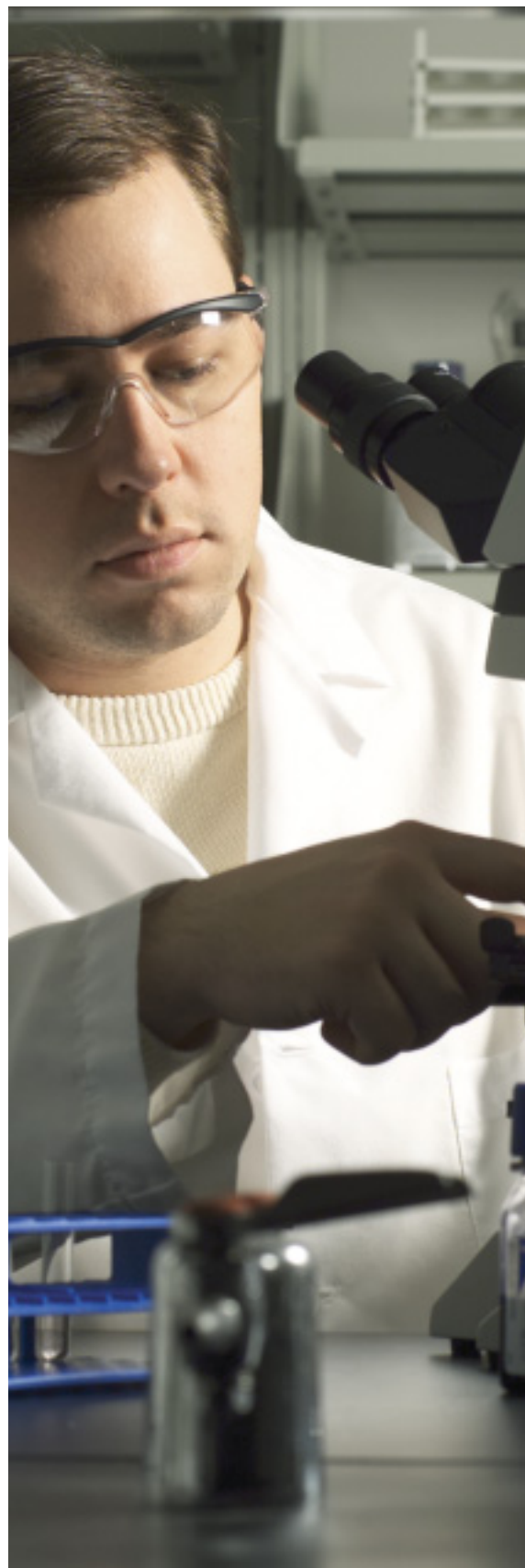
Morphology

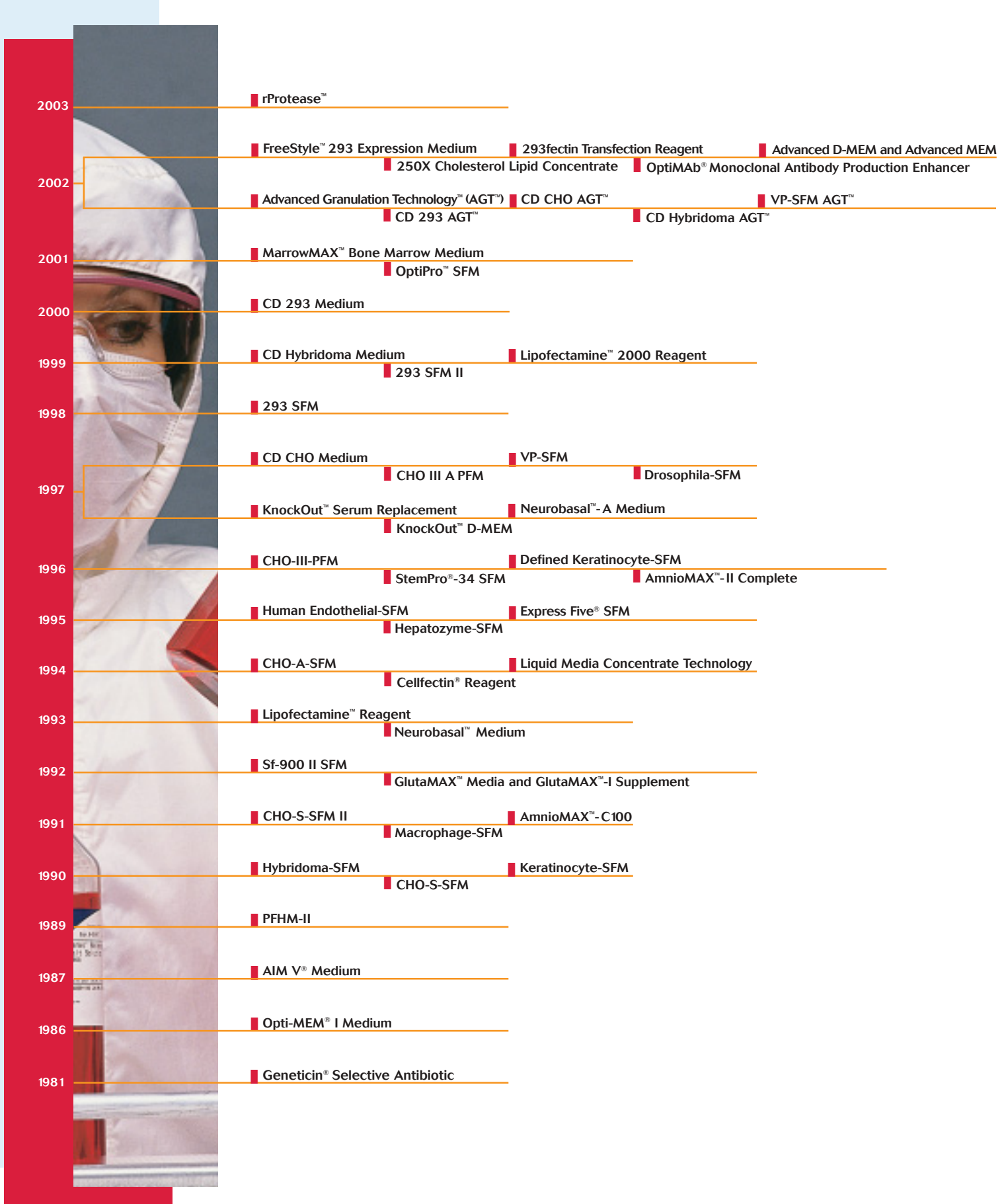
It is not uncommon to see slight changes in cellular morphology during and after adaptation to SFM. As long as doubling times and viability remain good, slight changes in morphology should not be a reason for concern.

Contact us to learn more.

Boost productivity, meet regulatory requirements, and simplify purification with GIBCO® serum-free cell culture.

To begin, go to www.invitrogen.com/gibco or call Cell Culture Technical Services, 1-800-955-6288.







United States Headquarters

Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, California 92008
Phone: 1 760 603 7200
Toll Free: 1 800 955 6288
Toll Free Fax: 1 800 331 2286
E-mail: tech_service@invitrogen.com

European Headquarters

Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
E-mail: eurotech@invitrogen.com

International Offices

Argentina 5411 4556 0844
Australia 1 800 331 627
Austria 0800 20 1087
Belgium 0800 14894
Brazil 0800 11 0575
Canada 800 263 6236
China 10 6849 2578
Denmark 80 30 17 40
France 0800 23 20 79
Germany 0800 083 0902
Hong Kong 2407 8450
India 11 577 3282
Italy 02 98 22 201
Japan 03 3663 7974
The Netherlands 0800 099 3310
New Zealand 0800 600 200
Norway 22 83 16 00
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Taiwan 2 2651 6156
For other countries see our website.

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