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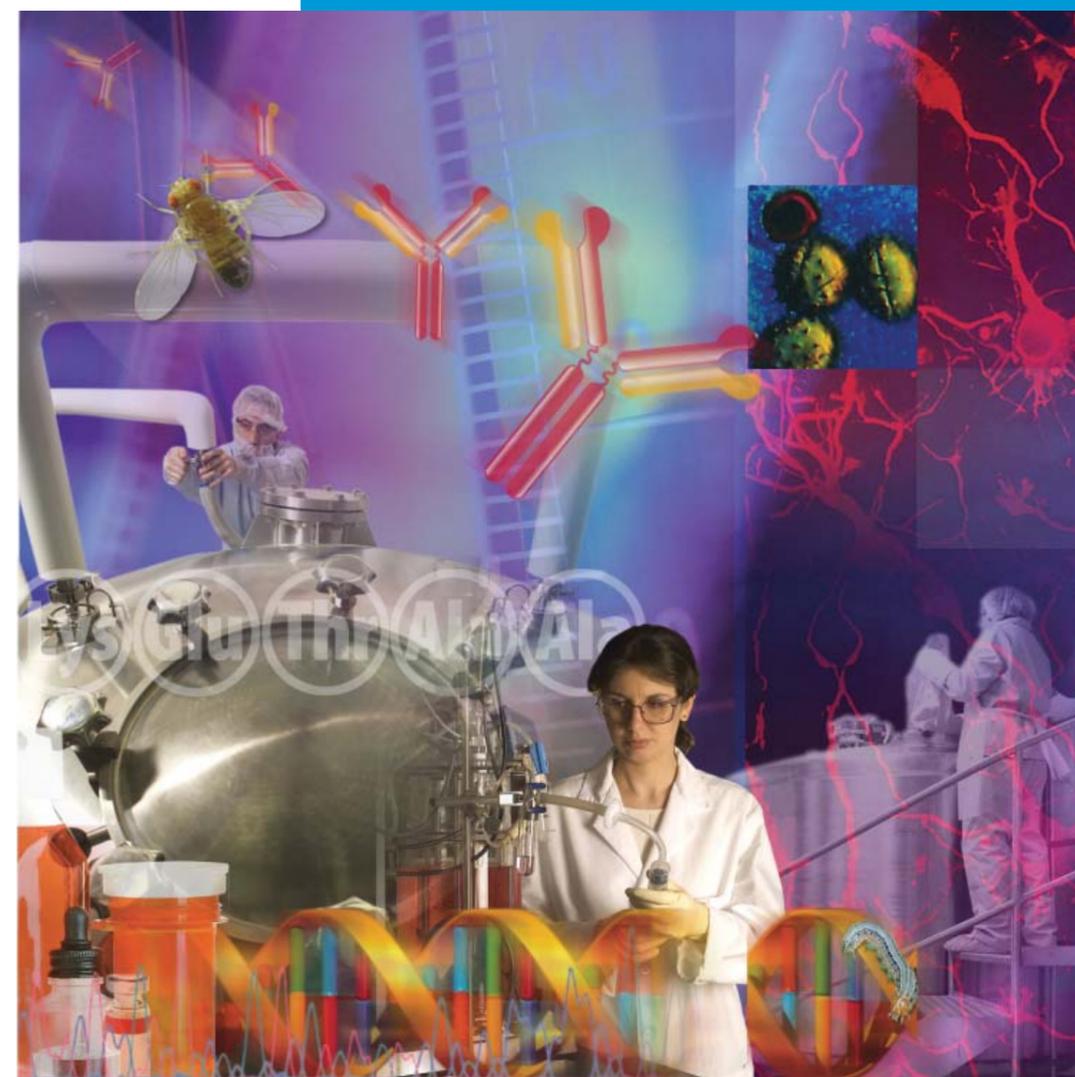
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A Guide to Serum-Free Cell Culture



www.invitrogen.com



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These products are for research use, and where appropriate, as raw material components in further cell culture manufacturing applications. They are not intended for human or animal diagnostic, therapeutic, or other clinical uses, unless otherwise stated.

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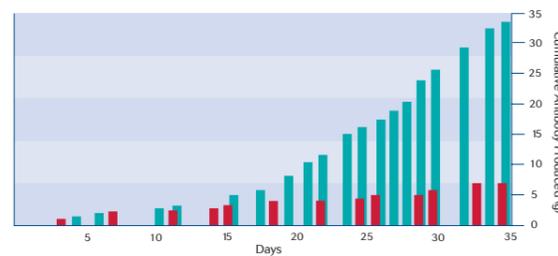
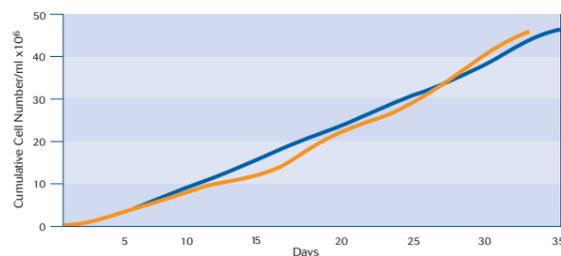
**Boost productivity,
meet regulatory requirements,
and simplify purification.**

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 centre of this guide to see which GIBCO™ medium is right for your particular cell line. Custom products and packaging are also available.

SFM

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™ products that are animal-origin-free are noted on the chart in the centre 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™ catalogue 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 litres to > 30,000 litres, or > 100,000 litres in dry format. In addition, we offer large media bag packaging options up to 500 litres.

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 Drug Master Files for several products.



GIBCO™ Serum-Free Media and Pre-Adapted Cells

Product	Optimized For	Applications	Size	Catalogue 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.	1,000 ml	11279-023
 CD Hybridoma AGT™†† Dry granular format of CD Hybridoma Medium.			1 X 1 L 1 X 10 L	12372-025 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 1,000 ml	12045-084 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.	500 ml 10 X 500 ml	12040-051 12040-085

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
 CD CHO Medium AGT™†† Dry granular format of CD CHO Medium.			1,000 ml 1 X 1 L 1 X 10 L	10743-029 12490-017 12490-025
CD CHO-A Medium	Adherent CHO cells		500 ml 1,000 ml	097-0182DJ 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 1,000 ml	12052-114 12052-098
 CHO III PFM†	Suspension CHO cells (including CHO-S cells)	Growth and production of recombinant proteins in suspension culture.	500 ml 1,000 ml	096-0334DJ 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 X 10 ⁷ cells	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
 CD 293 AGT™†† Dry granular format of CD 293 Medium.			1 X 1 L 1 X 10 L	12529-020 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.	1,000 ml	11686-029
 FreeStyle™ 293 Expression Medium	Suspension 293-F cells	Growth and transfection in suspension culture.	1,000 ml 6 X 1,000 ml	12338-018 12338-026
293-F Cells, Adapted to CD 293 Medium and 293 SFM II		7.5 X 10 ⁶ cells	1.5 ml	11625-019
293-H Cells, Adapted to CD 293 Medium and 293 SFM II		7.5 X 10 ⁶ cells	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
 VP-SFM AGT™†† Dry granular format of VP-SFM			1 X 1 L 1 X 10 L	12559-027 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 1,000 ml	10797-017 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 1,000 ml	10902-096 10902-088
D.Mel-2 Cells, Adapted to Drosophila SFM		1.5 X 10 ⁷ cells	1.5 ml	10831-014
High Five™ Cells, Adapted to Express Five® SFM		3 X 10 ⁶ cells	1.0 ml	B855-02
Sf9 Cells, Adapted to Sf-900 II SFM		1.5 X 10 ⁷ cells	1.5 ml	11496-015
Sf21 Cells, Adapted to Sf-900 II SFM		1.5 X 10 ⁷ cells	1.5 ml	11497-013

GIBCO™ Serum-Free Media and Pre-Adapted Cells

Product	Optimized For	Applications	Size	Catalogue No.
Neuronal Cell Culture				
Neurobasal™ Medium	Foetal 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)		Study growth of CNS progenitor or stem cells.	10 ml	098-0153SA
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
			1,000 ml	12055-083
AIM V® Medium, liquid Therapeutic Grade.		Growth of tumor infiltration lymphocytes (TIL cells), cytotoxic T-cells, and monocytes.	1,000 ml 10 L	087-0112DK 087-0112BK
Macrophage-SFM	Macrophages, monocytes	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
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 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	17005-075
Keratinocyte-SFM (with EGF, BPE, without CaCl₂)†			500 ml	37010-022
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

Contact Cell Culture Technical Services: eurotech@invitrogen.com, for more information.

† Drug Master File available

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, e-mail: eurotech@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.

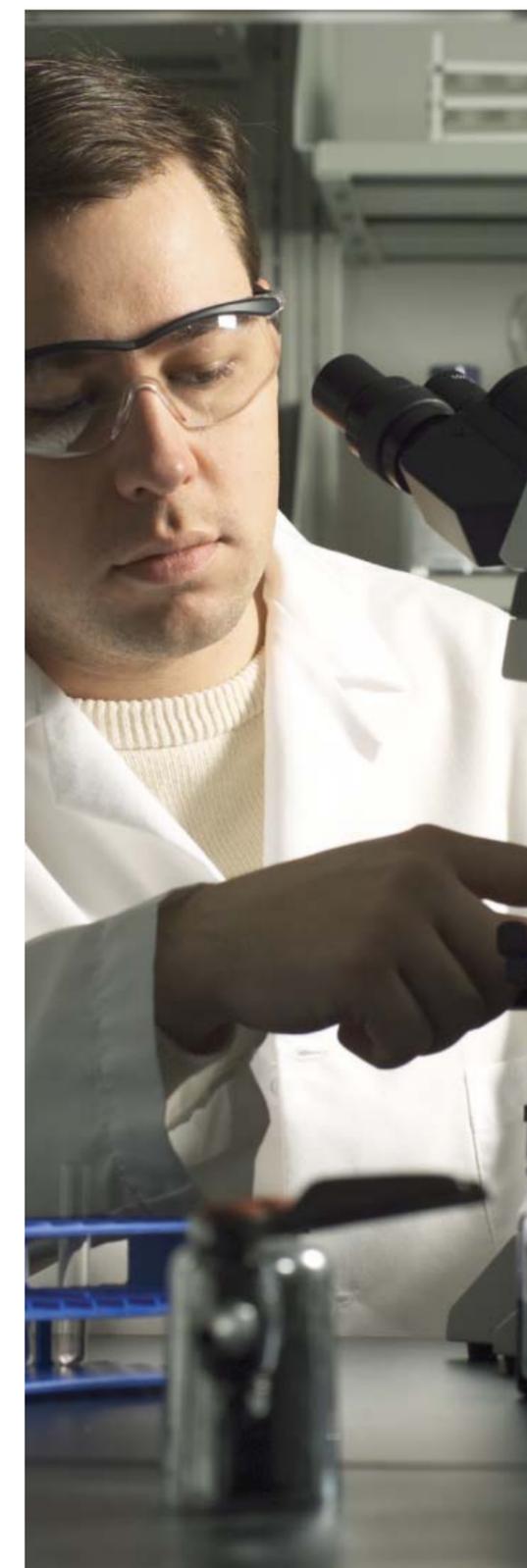
Morphology

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 contact Cell Culture Technical Services: eurotech@invitrogen.com



Innovation is Our Tradition

2003

rProtease™

2002

FreeStyle™ 293 Expression Medium 293fectin™ Transfection Reagent Advanced D-MEM and Advanced MEM
250X Cholesterol Lipid Concentrate OptiMAB® Monoclonal Antibody Production Enhancer

Advanced Granulation Technology™ (AGT™) CD CHO AGT™ VP-SFM AGT™
CD 293 AGT™ CD Hybridoma AGT™

2001

MarrowMAX™ Bone Marrow Medium
OptiPro™ SFM

2000

CD 293 Medium

1999

CD Hybridoma Medium Lipofectamine™ 2000 Reagent
293 SFM II

1998

293 SFM

1997

CD CHO Medium CHO III A PFM VP-SFM Drosophila-SFM

KnockOut™ Serum Replacement Neurobasal™ -A Medium
KnockOut™ D-MEM

1996

CHO-III-PFM Defined Keratinocyte-SFM
StemPro®-34 SFM AmnioMAX™-II Complete

1995

Human Endothelial-SFM Express Five® SFM
Hepatozyme-SFM

1994

CHO-A-SFM Liquid Media Concentrate Technology
Cellfectin® Reagent

1993

Lipofectamine™ Reagent
Neurobasal™ Medium

1992

Sf-900 II SFM
GlutaMAX™ Media and GlutaMAX™-I Supplement

1991

CHO-S-SFM II AmnioMAX™ - C100
Macrophage-SFM

1990

Hybridoma-SFM Keratinocyte-SFM
CHO-S-SFM

1989

PFHM-II

1987

AIM V® Medium

1986

Opti-MEM® I Medium

1981

Geneticin® Selective Antibiotic

