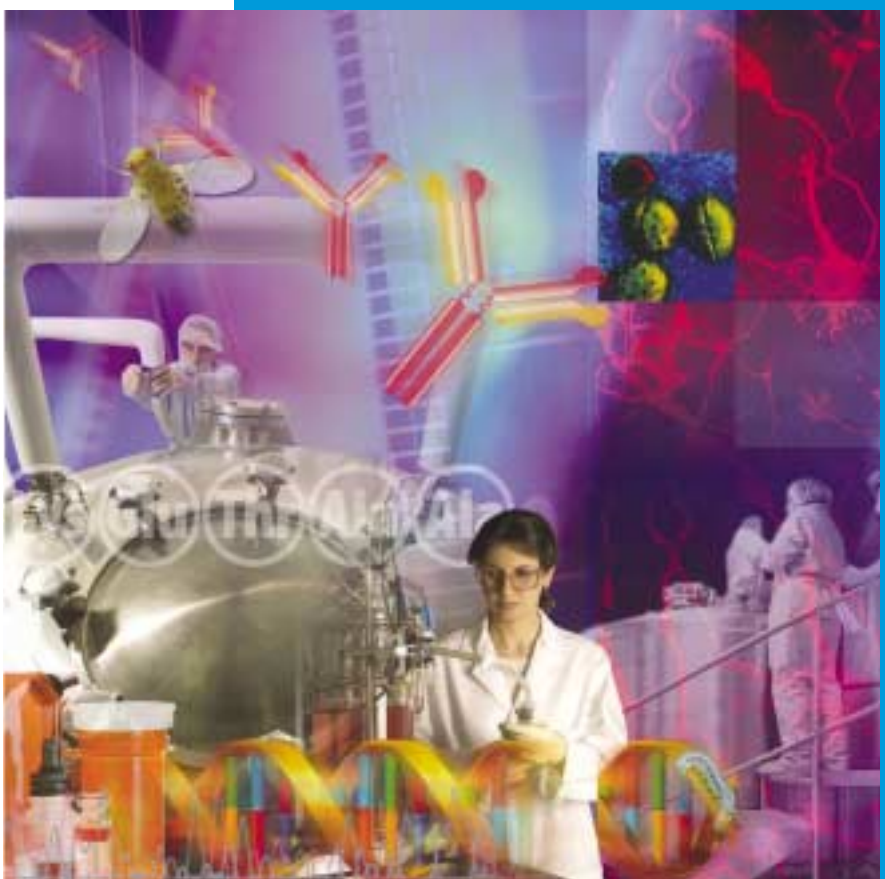
# A Guide to Serum-Free Cell Culture



GIBCO®
cell culture

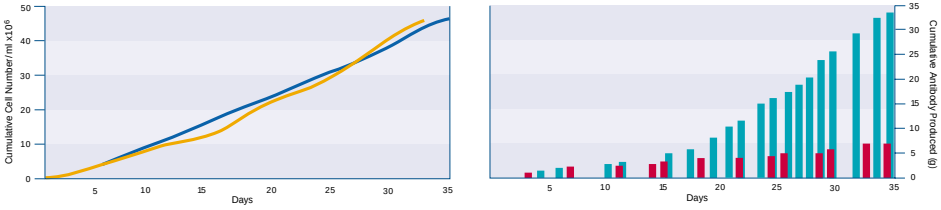
# Boost productivity, meet regulatory requirements, and simplify purification.

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 (■,■) or D-MEM with FBS (■,■) (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.

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 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 capablities 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® Media and Pre-Adapted Cells for Serum-Free Cell Culture

| 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<br>1,000 ml | 11279-015<br>11279-023 |
| **CD Hybridoma AGT™†** Dry granular format of CD Hybridoma Medium. | | | 1 × 1 L<br>1 × 10 L | 12372-025<br>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<br>1,000 ml | 12045-084<br>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<br>1,000 ml | 10743-011<br>10743-029 |
| **CD CHO Medium AGT™†** Dry granular format of CD CHO Medium. | | | 1 × 1 L<br>1 × 10 L | 12490-017<br>12490-025 |
| **CD CHO-A Medium** | Adherent CHO cells | | 500 ml<br>1,000 ml | 097-0182DJ<br>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<br>1,000 ml | 12052-114<br>12052-098 |
| **CHO III PFM†** | Suspension CHO cells (including CHO-S cells) | Growth and production of recombinant proteins in suspension culture. | 500 ml<br>1,000 ml | 096-0334DJ<br>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 |
| **CD 293 AGT™†** Dry granular format of CD 293 Medium. | | | 1 × 1 L<br>1 × 10 L | 12529-020<br>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<br>6 × 1,000 ml | 12338-018<br>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 × $10^7$ 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 × 1 L<br>1 × 10 L | 12559-027<br>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 *Drosophila melanogaster* cells (D.Mel-2, Schneider S2 cells) | Growth and maintenance medium for adherent or suspension culture. | 500 ml<br>1,000 ml | 10797-017<br>10797-025 |
| **Express Five® SFM** | Suspension BTI-TN-5B1-4 insect cells | Growth and maintenance of cells used for the baulovirus 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, (*Spodoptera frugiperda*), TN368 cells (*Trichoplusia ni*) | Growth and maintenance of cells used for BEVS for adherent or suspension culture. Large-scale production of recombinant protein expressed by BEVS. | 500 ml<br>1,000 ml<br>10 L | 10902-096<br>10902-088<br>10902-070 |
| **D.Mel-2 Cells, Adapted to Drosophila SFM** | | | 1.5 ml | 10831-014 |
| **High Five™ Cells, Adapted to Express Five® SFM** | | | 3 × $10^6$ 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 |

Contact Cell Culture Technical Services, 1-800-955-6288, for more information.

† Drug Master File available

| Product | Optimized For | Applications | Size | Catalog No. |
|---|---|---|---|---|
| **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)** | | 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 | *Ex vivo* activation of cytotoxic lymphocytes with IL-2 supplementation. | 500 ml<br>1,000 ml | 12055-091<br>12055-083 |
| **AIM V® Medium, liquid** Therapeutic Grade. | | Growth of tumor infiltration lymphocytes (TIL cells), cytotoxic T-cells, and monocytes. | 1,000 ml<br>10 L | 087-0112DK<br>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 *in vitro* toxicology. | 500 ml | 17005-042 |
| **Keratinocyte-SFM (with EGF, BPE, without $CaCl_2$)†** | | | 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**

# 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.

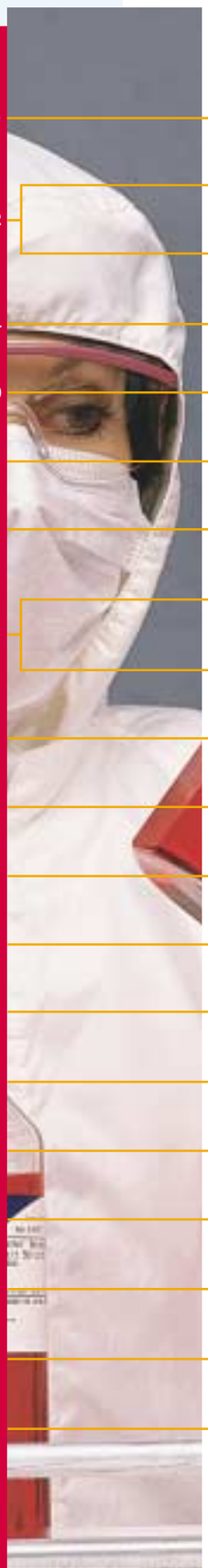
**Morphology**
It is not uncommon to see slight changes in cellular morphology during and after adaptation to SFM. As long as doubling times and viability remain good, slight changes in morphology should not be a reason for concern.

## 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.**

# 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
- VP-SFM
- CHO III A PFM
- 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

**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
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Hong Kong 2407 8450
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Italy 02 98 22 201
Japan 03 3663 7974
The Netherlands 0800 099 3310
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Norway 22 83 16 00
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For other countries see our website.

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