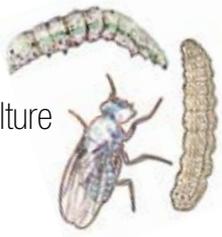
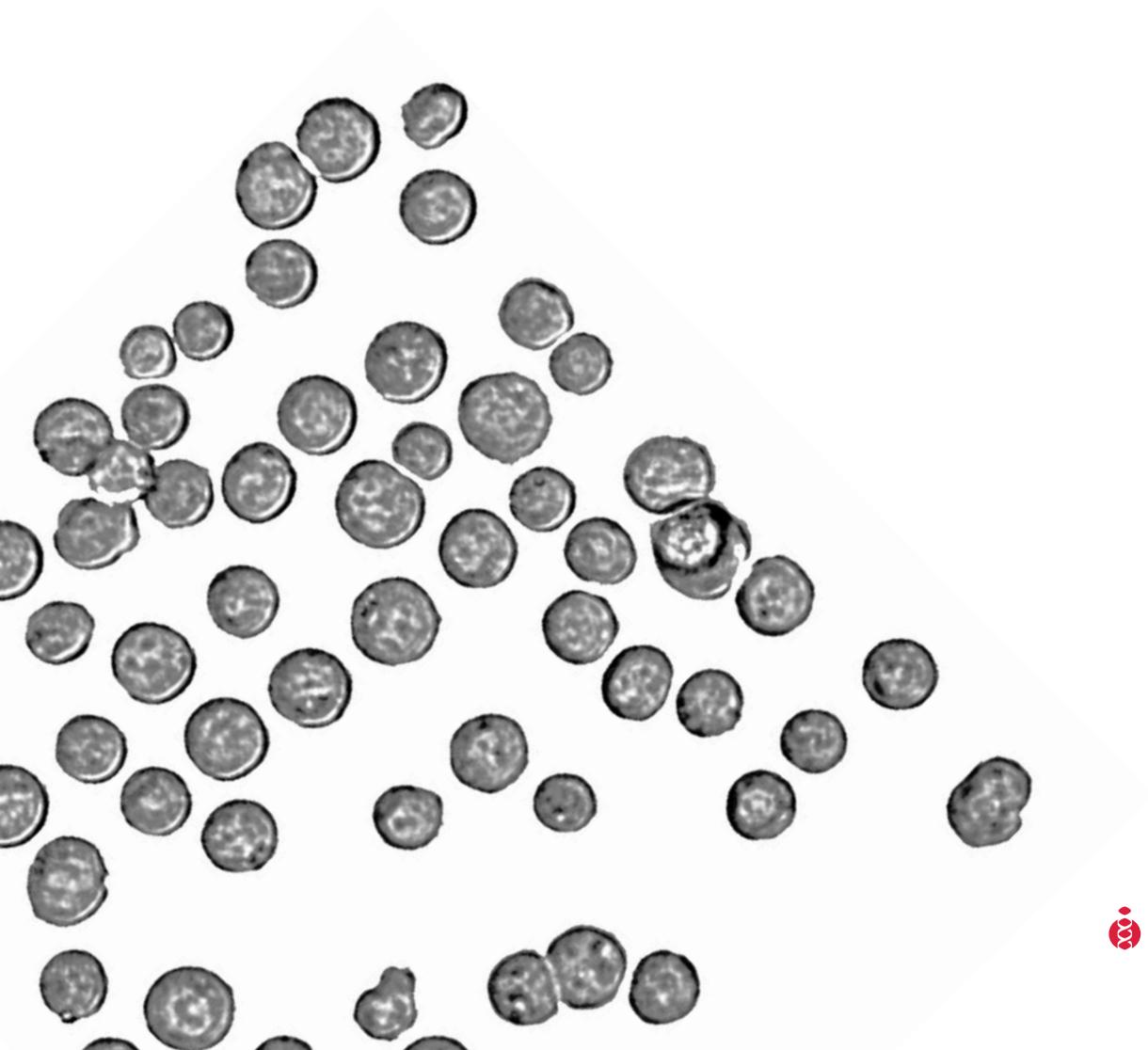
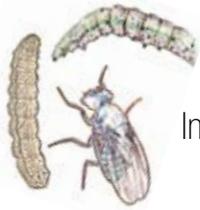


Insect Cell Culture



Enhance serum-free insect cell culture with GIBCO® Media





Quality media and adapted cell lines for optimal insect cell culture

- Maximize cell growth and recombinant protein yields
- Protein-free formulations optimized for specific cell types
- Convenient preadapted cells save time

Rely on GIBCO® media from Invitrogen for maximum growth and protein yields, trouble-free scale-up, and animal origin-free options. Select one of these ready-made media (Table 1), or talk to us about custom formulations and special packaging requirements.

Table 1—GIBCO® insect cell culture media.

Product	Optimized for	Applications	Protein free	Serum free	Animal origin free	Preadapted cell lines available
Sf-900™ III SFM	Suspension Sf9, Sf21 cells (<i>Spodoptera frugiperda</i>)	Growth and maintenance of cells used for the baculovirus expression vector system (BEVS) for adherent or suspension culture. Large-scale production of recombinant proteins expressed by BEVS	√	√	√	Sf9 Cells (Cat. no. 12659-017) Sf21 Cells (Cat no. 12682-019)
Drosophila-SFM	Suspension <i>Drosophila melanogaster</i> cells (D.Mel-2, Schneider S2 cells)	Growth and maintenance medium for adherent or suspension culture	√	√		D.Mel-2 Cells (Cat. no. 10831-014)
Express Five® SFM	Suspension BTI-TN-5B1-4 insect cells	Growth and maintenance of cells used for BEVS for adherent or suspension culture. Large-scale production of recombinant proteins expressed by BEVS	√	√		High Five™ Cells (Cat. no. B855-02)
Sf-900™ II SFM*	Suspension Sf9, Sf21 cells (<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 proteins expressed by BEVS	√	√		Sf9 Cells (Cat. no. 11496-015) Sf21 Cells (Cat. no. 11497-013)

* Drug Master File available.

Note: Cell lines from different sources, and different clones of the same cell line, may have highly specific nutritional requirements and may therefore prefer one medium over another. More than one medium formulation (if available) should be evaluated to determine the best option.

Sf-900™ III SFM

Animal origin-free/protein-free formulation

GIBCO® Sf-900™ III SFM is an animal origin-free and protein-free medium formulated with a low hydrolysate concentration to greatly reduce variability in performance (Figure 1). It is optimized to support and maximize Sf9 and Sf21 cell growth in suspension applications, as well as recombinant gene expression in both baculovirus and stable insect expression systems. This medium will support Sf9 cell growth to densities of $10\text{--}14 \times 10^6$ cells/ml.

Sf9 and Sf21 Cells

For your convenience, we offer Sf9 and Sf21 cells that are preadapted to suspension growth in Sf-900™ III SFM and commonly used for expression of recombinant proteins using the baculovirus expression vector system (BEVS). Sf9 is a clonal isolate derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf21-AE. Sf21 is an isolate from *S. frugiperda* ovarian cells.

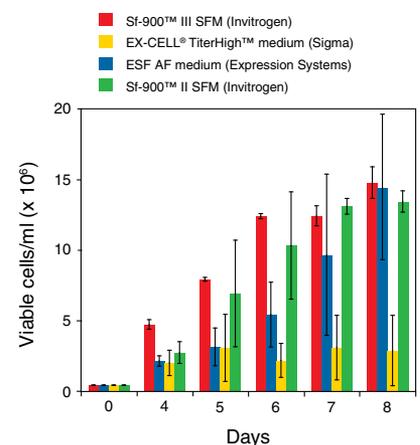
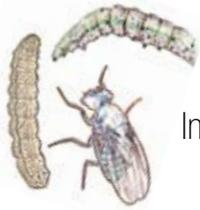


Figure 1—Sf9 cell growth in Sf-900™ III SFM compared to other insect cell culture media. Sf9 cells were sequentially adapted to all three tested animal origin-free (AOF) media (Sf-900™ III SFM, EX-CELL® TiterHigh™ medium, ESF AF medium) and Sf-900™ II SFM. Once the cells were adapted to each test medium (a total of 8 passages post-recovery), growth curves were determined by seeding 30 ml of medium in 125 ml shake flasks at a seeding density of 4×10^5 viable cells/ml. Each test condition represents the average of two lots of the respective medium with two replicates each (a total of 4 replicates per test condition).



Insect Cell Culture

Drosophila-SFM

Protein-free formulation

Optimized for the growth of *Drosophila melanogaster* (D.Mel) cells, protein-free Drosophila-SFM outperforms other commercially available media for culturing D.Mel-2 cells, with suspension cultures reaching peak cell densities of $>10^7$ cells/ml. Cells grown in other media adapt quickly and easily to this medium. The absence of protein from FBS supplementation promotes easier downstream purification of functional protein. D.Mel cells can be transfected for stable or transient expression of recombinant proteins. To prevent lipid interference, transfection of D.Mel cells is best performed in Drosophila-SFM.

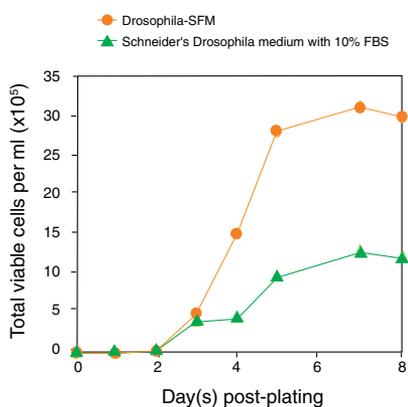


Figure 2—Growth of *D. melanogaster* cells in Drosophila-SFM. Early-passage *D. melanogaster* cells were seeded at a density of 3×10^5 viable cells per ml in 35 ml medium in 125 ml shake flasks. The flasks were incubated at 27°C and rotated at 135 rpm. Samples were taken daily and evaluated for viable cell density with the use of trypan blue. The cells grown in Drosophila-SFM reached significantly higher densities than cells in Schneider's Drosophila medium supplemented with 10% FBS. Maximal growth rates were found to be during days 3 and 4.

D.Mel-2 Cells

For your convenience, we offer D.Mel-2 cells preadapted to Drosophila-SFM. These cells are isolated from late-stage *D. melanogaster* embryos and are used for transient or stable expression of recombinant proteins (Figure 2).

Cellfectin® Reagent for high-efficiency insect transfection

Cellfectin® Reagent is formulated and tested for optimal transfection of insect cells. Transfection with Cellfectin® Reagent leads to consistent and efficient transfection of D.Mel-2, S2, Sf9, Sf21, and High Five™ cells when using BEVS or stable insect expression plasmids (Figure 3).

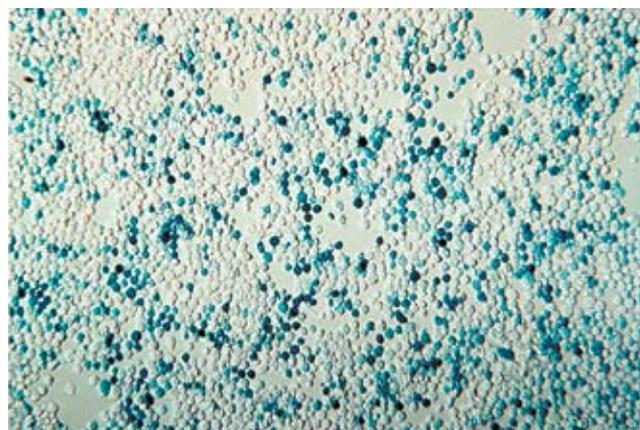


Figure 3—Cellfectin® Reagent for transfection of insect cells. 9×10^5 Sf9 cells/well were plated in Sf-900™ II SFM in a 6-well plate. One hour later, the cells were transfected with bacmid DNA coding for the β -glucuronidase gene, using Cellfectin® Reagent. The cells were stained with X-glucuronidase 72 hr post-transfection to detect β -gluc expression.

BaculoDirect™ Baculovirus Expression System

The BaculoDirect™ Baculovirus Expression System is the fastest and easiest method for generating recombinant baculovirus. The BaculoDirect™ system utilizes the strong polyhedrin promoter for high-level expression and a counter-selection cassette for easy isolation of parent-free viral stock without the need for plaque purification. From the initial transfection to the isolation of the baculovirus stock, the BaculoDirect™ system requires only eight hours of hands-on time—less than half the time required with traditional baculovirus systems.

Sf-900™ II SFM

Protein-free formulation

GIBCO® Sf-900™ II SFM is a protein-free medium optimized to support and maximize Sf9 and Sf21 cell growth in suspension applications, as well as recombinant gene expression in both baculovirus and stable insect expression systems. This medium provides excellent results in the production of a variety of recombinant proteins (Figures 4 and 5) and will support Sf9 cell growth to densities of 8–10 × 10⁶ cells/ml.

Sf9 and Sf21 Cells

For your convenience, we offer Sf9 and Sf21 cells that are preadapted to suspension growth in Sf-900™ II SFM and commonly used for expression of recombinant proteins using BEVS.

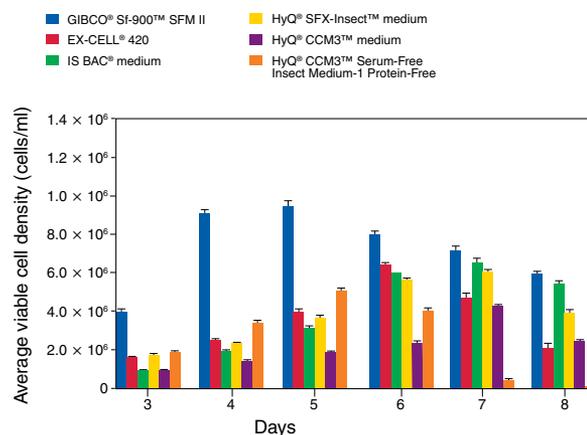


Figure 4—Growth of Sf9 cells expanded in TNM-FH medium plus 10% FBS, then adapted to various test media. After 15 passages in the test media, growth curves were determined by seeding 30 ml of medium in 125 ml shake flasks (8 replicates per medium) at 400,000 cells/ml, and counting viable cell number on each day post-seed.

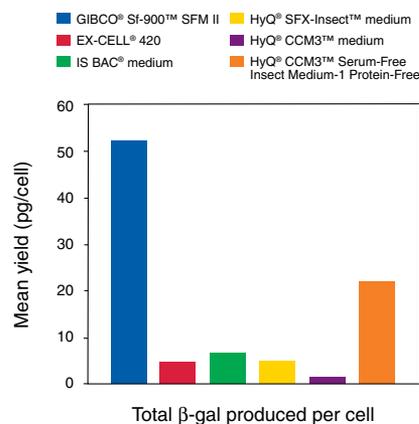


Figure 5— Protein production from Sf9 cells expanded in TNM-FH medium plus 10% FBS, then adapted to various test media. After 13 passages in the test media, 30 ml of medium in 125 ml shake flasks (3 replicates per medium) were seeded at 600,000 cell/ml, incubated for 24 hr, then infected at MOI = 3 with AcMNPV expressing a recombinant β-galactosidase. Samples were taken daily and counted for viable cells and assayed for β-gal activity (combined media and cell samples). The maximum β-gal production per ml (on day 4 or 5 post-infection) was divided by the maximum viable cell density observed post-infection, to obtain a per-cell β-gal productivity value.



Insect Cell Culture

Custom 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. Media are available in different formats for easy scale-up to meet the needs of various levels of product development: R&D, process development, pilot plant, and manufacturing. We can produce volumes as small as a few liters to >30,000 L, or >100,000 L in dry format. In addition, we offer large media bag packaging options up to 500 L. The Custom Product Services team can also assess feasibility and provide options for formulation design, testing, and packaging for your proprietary formulations.

For information, call your regional customer service representative or visit us at www.invitrogen.com/GIBCO.

References

Weiss, S.A., et al. (1993) *Insect Cell Culture Engineering* (Goosen, M.F.A., Daugulis, A.J., and Faulkner, P., eds.) Marcel Dekker, Inc., NY.

Weiss, S. A., et al. (1995) *Methods in Molecular Biology* (Richardson, C.D., ed.) 39, Humana Press, Totowa, NJ.

SF-900™ II SFM

Godwin, G. and Whitford, W. (1993) *FOCUS*® 13, 44.

Express Five® SFM

Godwin, G., et al. (1995) *FOCUS*® 17, 58.

Ordering Information

Product	Quantity	Cat. no.
Sf-900™ III SFM (1X), liquid (contains L-glutamine)	500 ml	12658-019
	1,000 ml	12658-027
	10 L	12658-035
Sf9 Cells Adapted in Sf-900™ III SFM	1.5 ml	12659-017
Sf21 Cells Adapted in Sf-900™ III SFM	1.5 ml	12682-019
Drosophila-SFM (1X), liquid	500 ml	10797-017
	1,000 ml	10797-025
D.Mel-2 Cells	1.5 ml	10831-014
Express Five® SFM (1X), liquid	1,000 ml	10486-025
High Five™ Cells	3 × 10 ⁶ cells/ml	B855-02
Sf-900™ II SFM (1X), liquid (contains L-glutamine)	500 ml	10902-096
	1,000 ml	10902-088
	6 × 1,000 ml (case)	10902-104
	5 L	10902-161
	10 L	10902-179
Sf-900™ II SFM (1X), liquid (w/o Met and Cystine) (contains L-glutamine)	500 ml	21012-026
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
Sf-900™ Medium (1.3X), liquid (contains L-glutamine)	100 ml	10967-032
Cellfectin® Reagent	1 ml	10362-010
BaculoDirect™ Baculovirus Expression Kit	5 rxns	12562-013
BaculoDirect™ Baculovirus Transfection Kit	5 rxns	12562-039

Protein-free media

GIBCO® protein-free media contain no proteins, but may contain plant or yeast hydrolysates. Many are animal origin free.

Serum-free media

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

Animal origin-free media

GIBCO® animal origin-free media contain no material directly derived from animal tissues, cells, or body fluids of higher eukaryotic organisms, such as mammals, fish, birds, insects, etc. The term “animal origin” does not pertain to eukaryotic organisms such as the higher plants, fungi, protozoa, and algae, nor does it include prokaryotic organisms such as bacteria and blue-green algae.