









## Optimize Serum-Free HEK 293 Cell Culture with GIBCO® Media

- Choose from chemically-defined, protein-free, and serum-free formulations
- Animal-origin-free products minimize risk
- Convenient pre-adapted 293-F, 293-H, and FreeStyle™ 293-F cells save time
- Custom products and packaging available
- Easy to scale up to large volume cultures

Product	Optimized For	Applications
 CD 293 Medium†	Suspension 293 cells (including 293-F, 293-H)	Growth and recombinant protein or adenovirus production in suspension culture.
 CD 293 AGT™†† Dry granular format of CD 293 Medium.		
 293 SFM II† Low-protein < 10µg/ml.	Suspension 293 cells, HeLa S3 cells	Growth and recombinant protein or adenovirus production in suspension culture.
 FreeStyle™ 293 Expression Medium	Suspension 293-F cells	Growth and transfection in suspension culture.
293-F Cells, Adapted to CD 293 Medium and 293 SFM II		
293-H Cells, Adapted to CD 293 Medium and 293 SFM II		
FreeStyle™ 293-F Cells, Adapted to FreeStyle™ 293 Expression Medium		

 Chemically-Defined Media    
  Serum-Free Media    
  Pre-Adapted Cells    
  Animal-Origin-Free Product

† 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.

### CD 293 Medium

#### Chemically-Defined Formulation for High-Density Suspension Culture

GIBCO® CD 293 Medium is a chemically-defined, protein-free medium optimized for growth and recombinant protein or adenovirus production of 293 cells (including 293-F, 293-H)

in suspension culture. It contains no components of animal origin. It is formulated without L-glutamine, providing added stability.



**Figure 1. The presence of a proprietary cellular dispersant in CD 293 Medium prevents the aggregation of 293 cells.** Cells were cultured in 20 ml CD 293 Medium (panel A) in 125 ml shaker flasks and compared to D-MEM+ 10% FBS (panel B), or another commercially available 293 medium (panel C). All experiments were conducted at 37°C in a humidified 92% air, 8% CO<sub>2</sub> environment at an agitation rate of 130 rpm. Resultant photographs are from day three cultures, fourth passage.

## 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.

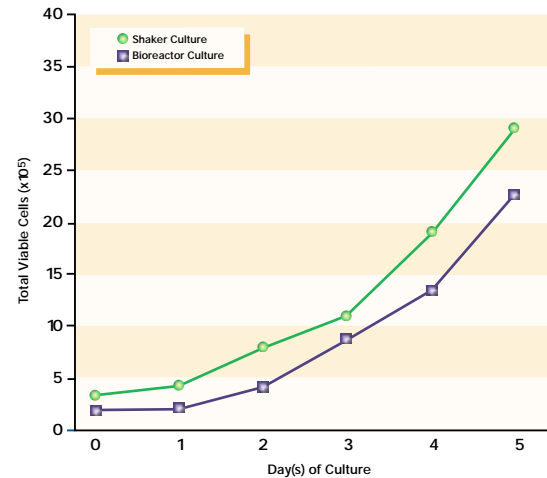
## CDM

### 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.

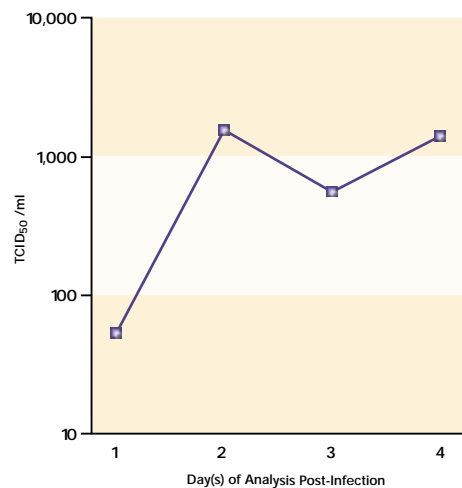
- Completely defined system eliminates variability
- Consistent performance improves reproducibility
- Decrease possibility of contamination by adventitious agents
- Save time with simplified purification and downstream processing

### Scalability of HEK 293 Cells from Shaker Flasks to Bioreactors



**Figure 2. The average population doubling time for 293 cells in bioreactor system is approximately 24 hours.** Shaker flasks: Average initial seeding density was  $3 \times 10^5$  cells/ml ( $n=30$ ) in 125-ml shaker flasks. Flasks were maintained at 37°C with 8% CO<sub>2</sub> and 92% air, and an agitation rate of 130 rpm. CelliGen® bioreactor: Average initial seeding density was  $2 \times 10^5$  cells/ml ( $n=5$ ) in a 5-L bioreactor containing 3.8 L media at 37°C; impeller speed, 100 rpm. Cell cultures were evaluated daily for five days.

### Adenovirus Type-5 Yield by HEK 293 Suspension Cells in CD 293 Medium



**Figure 3. Optimal yields of adenovirus-5 appear to be approximately 48 hours post-infection.** Cells adapted to CD 293 Medium were cultured at 37°C in a humidified 95% air and 5% CO<sub>2</sub> environment. Adenovirus was added to the cultures ( $2 \times 10^6$  cells/ml) at a MOI= 10 for a period of two hours. Viral infectivity measurements were conducted on daily samples for four days. Viral titer is expressed as 50% tissue culture infectious dose (TCID<sub>50</sub>/ml).

CD 293 Medium contains a proprietary cellular dispersant demonstrated to reduce the natural tendency for 293 cells to form multicellular aggregates (figure 1). The medium supports the growth of 293 cells in bioreactors to maximize the production of adenovirus and/or recombinant proteins (figures 2 and 3).

CD 293 Medium is available in a ready-to-use (1X) liquid format and in a new, easy-to-use granular format. Advanced Granulation Technology™ (AGT™) media are complete, pH pre-adjusted and require only standard supplementation with L-glutamine or GlutaMAX™-I Supplement for L-glutamine dependent systems. AGT™ media can help to reduce total cycle costs, decreasing time involved in raw material planning, procurement, and testing, as well as media preparation.

## FreeStyle™ 293 Expression Medium

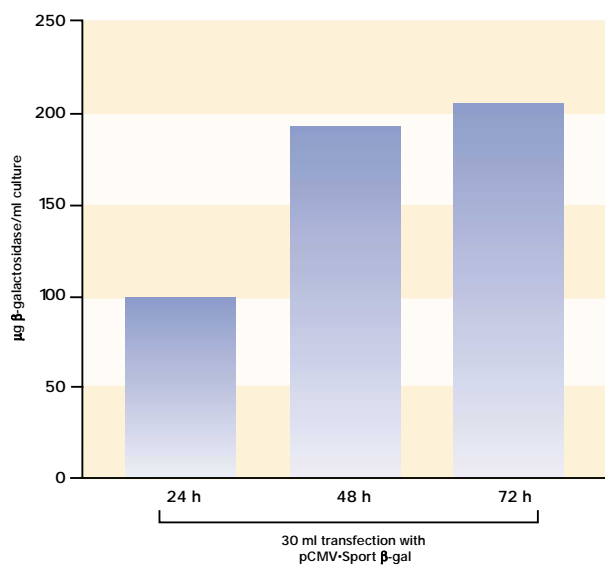
### Chemically-Defined Formulation for Growth and Transfection in Suspension Culture

FreeStyle™ 293 Expression Medium is a chemically-defined, protein-free medium specifically developed to support the growth and transfection of 293-F cells in suspension culture. This medium has been supplemented with GlutaMAX™-I Supplement, a stable form of L-glutamine. It is free of animal-origin components.

FreeStyle™ 293 Expression Medium encourages high levels of protein expression from FreeStyle™ 293-F cells (figure 4). Because it is protein-free, this medium saves time and effort in downstream purification and processing of recombinant proteins. In addition, it saves significant time and costs associated with adaptation of cell cultures to serum-free conditions.

This medium is also included in Invitrogen's FreeStyle™ 293 Expression System, which conveniently packages together 293fectin™ Transfection Reagent and pre-adapted FreeStyle™ 293-F cells.

### Protein Produced From a Transient FreeStyle™ Transfection



**Figure 4.** FreeStyle™ 293-F Cells were cultured in 30 ml of FreeStyle™ 293 Expression Medium to a density of  $1 \times 10^6$  cells/ml. Lipid:DNA complexes were prepared using 30 µg of pCMV-Sport β-gal DNA and 40 µl of 293fectin™. At the timepoints shown, cells were harvested and assayed for level of β-galactosidase protein. An untransfected control was performed in parallel and produced no measurable β-gal activity at any of the time points.

## 293 SFM II

### Serum-Free Formulation for High-Density Suspension Culture

293 SFM II is a serum-free, low-protein (< 10 µg/ml) medium optimized for high-density suspension culture of 293 cells. It is formulated without L-glutamine, providing added stability.

## 293-F and 293-H, and FreeStyle™ 293-F Cells

For your convenience, we offer 293-F and 293-H cells, pre-adapted to CD 293 Medium and 293 SFM II. The 293-F line is characterized by above-average growth rates. The 293-H line is easily converted to monolayer culture for adherent application. FreeStyle™ 293-F cells are pre-adapted to FreeStyle™ 293 Expression Medium.

## 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 liters, or > 100,000 liters in dry format. In addition, we offer large media bag packaging options up to 500 liters. In addition, we offer large media bag packaging options up to 500 liters.

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 1-800-955-6288, Ext. 46966.**

## Ordering Information

	Description	Catalog No.	Size
	CD 293 Medium (1X), liquid Requires supplementation with 4 mM L-glutamine or GlutaMAX™-I Supplement prior to use.	11913-019	1,000 ml
	CD 293 AGT™ Dry granular format of CD 293 Medium.	12529-020 12529-012	1 X 1 L 1 X 10 L
	FreeStyle™ 293 Expression Medium	12338-018 12338-026	1,000 ml 6 X 1,000 ml (case)
	293 SFM II (1X), liquid	11686-029	1,000 ml
	293-F Cells	11625-019	1.5 ml
	293-H Cells	11631-017	1.5 ml
	FreeStyle™ 293-F Cells	R790-07	1 X 10 <sup>7</sup> cells
<b>Related Products</b>			
<b>Nutritional Supplements</b>			
	GlutaMAX™-I Supplement Stable form of L-glutamine.	35050-061	100 ml
	L-Glutamine-200 mM (100X), liquid	25030-081	100 ml
<b>Selective Antibiotics</b>			
	Geneticin® Selective Antibiotic, powder	11811-031	5 g
	Geneticin® Selective Antibiotic, liquid 50 mg/ml.	10131-035 10131-027	20 ml 100 ml
<b>Transfection Reagents</b>			
	293fectin™ Transfection Reagent Transfects FreeStyle™ 293-F cells in serum-free suspension.	12347-019	1 ml
	Lipofectamine™ 2000 Reagent Transfects adherent 293 cells.	11668-027 11668-019	0.75 ml 1.5 ml
	Lipofectamine™ 2000 CD An animal-origin-free, chemically-defined version of Lipofectamine™ 2000.	12566-014	1 ml
<b>293 Expression System</b>			
	FreeStyle™ 293 Expression System Includes FreeStyle™ 293 Expression Medium, 293fectin™ Transfection Reagent, FreeStyle™ 293-F cells.	K9000-01	1 kit
<b>For use with CD 293 AGT™</b>			
	Distilled Water	15230-196 15230-162 15230-204 15230-147	20 X 100 ml (case) 500 ml 10 X 500 ml (case) 1,000 ml

All media listed above can be customized to suit your needs. Please inquire.



www.invitrogen.com



Corporate Headquarters: Invitrogen Corporation • 1600 Faraday Avenue • Carlsbad, California 92008 U.S.A.  
Tel: 1 760 603 7200 • Tel (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



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. © 2003 Invitrogen Corporation PAS03-041MS Part No. 332-032443A