CD 293 Medium

P R O D U C T   N E W S

- *Engineered for high-density suspension culture*
- *Minimizes introduction of adventitious agents*
- *Improves cell viability*
- *Provides easy adaptation*
- *Simplifies downstream production*

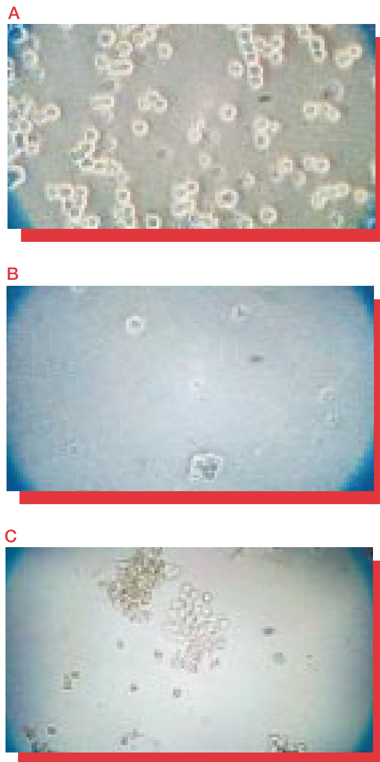

Figure 1. The presence of a proprietary cellular dispersant in CD 293 prevents the aggregation of 293 cells. Cells were cultured in 20 ml CD 293 Medium (panel A) in 125 ml shaker flasks and compared to D-MEM + 10% FBS (panel B), or a commercially available competitor's 293 medium (panel C). All experiments were conducted at 37˚C in a humidified 92% air, 8% CO_2 environment at an agitation rate of 130 rpm. Resultant photographs are from day three cultures, fourth passage.

HEK 293 cells were originally derived from human embryonic kidney (1) and subsequently demonstrated to be a useful cell type to produce adenovirus, and other viral vectors (2-4) and effectively glycosylated human recombinant proteins (5-7).

Monolayer culture and cell culture conditions using media formulated with serum-supplementation have historically been the choice for 293 cell cultivation. There are several problems inherent in this methodology. Adherent cell culture is more labor intensive and expensive as compared to suspension culture. The presence of serum can also be responsible for confounding factors in experimentation and subsequent data analysis. Lot-to-lot reproducibility of results can also be problematic. In addition, the use of serum in a cell culture medium may present process or regulatory concerns by introducing undefined components that could interfere with downstream purification and final product quality.

In response to the difficulties associated with traditional 293 cell culture, we have developed GIBCO™ CD 293 Medium, a chemically-defined, protein-free medium specifically engineered for high-density suspension culture of 293 cells.

CD 293 medium is devoid of any animal-origin components, undefined lysates, or hydrolysates. CD 293 Medium can be used for a number of applications including the propagation of viruses, production of recombinant proteins, and utilization in drug screening (8).

GIBCO™ 293 SFM II is manufactured without the addition of any human- or animal-origin components, and has a very low (10 mg/L) protein concentration.

- Protein-Free Formulation — Minimizes the introduction of adventitious agents and simplifies downstream purification.
- Improved Cell Viability — Contains a proprietary cellular dispersant demonstrated to reduce the natural tendency for 293 cells to form multicellular aggregates (*figure 1*).
- Easy Adaptation — Monolayer-dependent, adherent 293 cells adapt easily to growth in serum-free suspension using a simple protocol (*figure 2, reverse*).
- High-Density Growth — Supports growth of 293 cells in bioreactors to maximize the production of adenovirus and/or recombinant proteins (*figures 3 and 4, reverse*).
- Formulated without L-glutamine — Avoids problems associated with the degradation of L-glutamine, including ammonia toxicity. It is recommended that CD 293 Medium be supplemented with L-glutamine (4 mM) or GlutaMax™-I prior to use.

GIBCO™
Invitrogen Corporation

Typical 293 Cell Growth Curve in CD 293 Medium

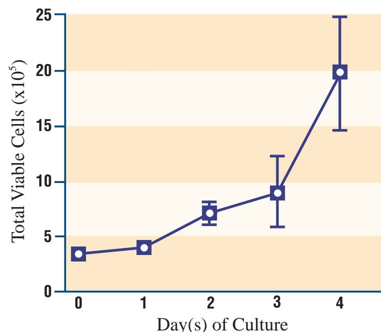

Figure 2. The average population doubling time for 293 cells in shaker flask format is approximately 33 hours. 293 HEK suspension cells were seeded at 3 × 10^5 cells/ml in 125 ml shaker flasks containing 20 ml medium. (Temperature, 37˚C; humidified atmosphere, 8% CO_2 and 92% air, and an agitation rate of 130 rpm.) Results derived from the mean of four different production lots of CD 293 Medium were compared in total (N=13-50). Results show average doubling time of 33 hours and average density approaching 2 × 10^6 over a period of four days.

Growth of 293 Cells in Shaker or Bioreactor System in CD 293 Medium

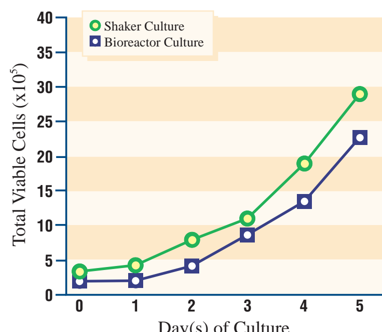

Figure 3. The average population doubling time for 293 cells in bioreactor system is approximately 24 hours. Shaker flasks: Average initial seeding density was 3 × 10^5 cells/ml (N=30) in 125-ml shaker flasks. Flasks were maintained at 37˚C with 8% CO_2 and 92% air, and an agitation rate of 130 rpm. CelliGen® bioreactor: Average initial seeding density was 2 × 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 293 HEK Suspension Cells in CD 293 Medium

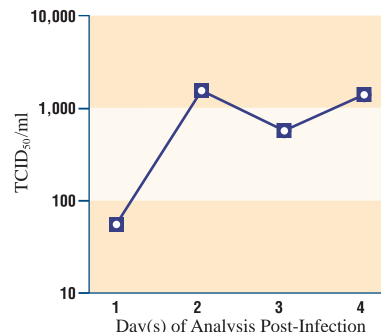

Figure 4. Optimal yields of adenovirus-5 appear to be approximately 48 hours post-infection. Cells adapted to CD 293 Medium were cultured at 37˚C in a humidified 95% air and 5% CO_2 environment. Adenovirus was added to the cultures (2 × 10^6 cells/ml) at a MOI=10 for a period of two hours. Viral infectivity measurements were conducted on daily samples for four days. Viral titer is expressed as 50% tissue culture infectious dose ($TCID_{50}$/ml).

Ordering Information

Description	Cat. No.	Size
CD 293 Medium *Requires supplementation with 4 mM L-glutamine or GlutaMax™-I Supplement prior to use.*	11913-019	1,000 ml
Related Products		
293 SFM II (1X), liquid	11686-011 11686-029	500 ml 1,000 ml
293-F Cells, SFM-adapted	11625-019	3 ml
293-H Cells, SFM-adapted	11631-017	3 ml
L-Glutamine-200 mM (100X), liquid*	25030-081	100 ml
GlutaMax™-I Supplement	35050-061	100 ml

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

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