GIBCO™

Enhanced CHO Production Systems

Improve suspension culture expansion, transfection, and protein production in DG44 cells

Several new kits for streamlining protein generation in CHO DG44 (dhfr⁻) cells are now available. The cells, media, and transfection reagents are designed for suspension culture, reducing the time needed to get to expressed protein. You'll easily create high density CHO suspension cultures, efficiently transfect in suspension, and express protein; all in chemically defined, animal component-free media systems.

Inherent selection with dhfr⁻cells

DG44 cells, dihydrofolate reductase deficient (dhfr⁻) derivates of Chinese Hamster Ovary (CHO) cells, are routinely used to establish cell lines for production of recombinant protein. They were developed by Dr. Chasin at Columbia University using gamma rays to eliminate the entire dhfr locus. In non-mutated cells, dhfr is an essential enzyme for de novo synthesis of glycine, purines, and thymidylate. This allows dhfr to be used as a dominant selectable marker and a gene amplifier for the expression of proteins in dhfr⁻ cell lines. The dhfr⁻ mutation in DG44 cells is stable and irreversible, which makes it a suitable mammalian cell line for production of recombinant proteins. GIBCO™ offers a complete system for the growth and expansion of DG44 (dhfr⁻) cells in suspension, including:

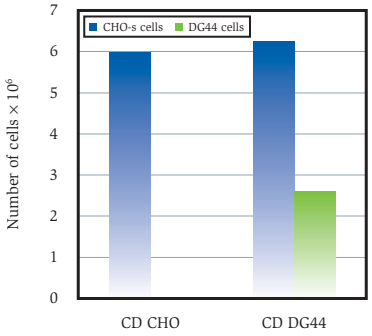
- **DG44 Cells and Media Kit**—fully characterized DG44 cells pre-adapted to chemically defined, animal origin component-free media for growth
- **DG44 Transfection Kit**—a complete kit optimized for high-efficiency transfection of CD44 cells in suspension
- **Growth Optimization Kits**—two kits that allow you to conveniently optimize growth conditions for your specific applications

Optimize Growth with the DG44 Cells and Media Kit

The DG44 Cells and Media Kit is optimized for suspension growth of CHO-DG44 (dhfr⁻) cells and expression of recombinant proteins in suspension culture. This kit provides characterized DG44 cells selected for superior cell growth and transfection efficiencies and CD DG44 Medium, an animal origin component-free, chemically defined formulation that contains no proteins, hydrosylates, or components of unknown composition (Table 1). CD DG44 Medium is supplemented with necessary components for the growth and maintenance of dhfr⁻ cell lines, including hypoxanthine and thymidine. GIBCO™ DG44 Cells are pre-adapted to this medium and grow in suspension culture with densities of $\sim$2-3 $\times$ 10^6 cells/ml. DG44 cells transfected with a plasmid carrying dhfr as a marker can be grown in this medium without the need for any selectable drug agents, providing a great advantage for the production of diagnostic or therapeutic recombinant proteins (Figures 1 and 2).

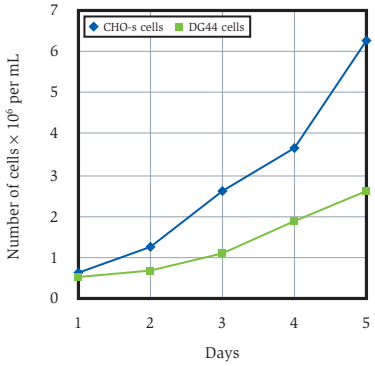
GIBCO™
cell culture

Figure 1—Comparison of CHO-s and DG44 cell growth in different media



Comparison of cell growth of CHO-s and DG44 cells in CD CHO or CD DG44 medium. Cell growth of CHO-s cells was equivalent in CD CHO medium without Hypoxanthine (HT) or CD DG44 medium supplemented with HT. DG44 cells did not survive in CD CHO medium without HT and died within 48 hrs. No dhfr (+) revertants were detected. DG44 cells require HT for cell growth and grow well in CD DG44 medium.

Figure 2—Comparison of CHO-s and DG44 cell growth over time



Comparison of cell growth rate of CHO-s and DG44 cells in CD DG44 medium. CHO-s and DG44 cells were seeded in CD DG44 medium at a density of 3×10^5 cells per mL. Compared to CHO-s cells, growth of DG44 cells was lower but the CD DG44 medium supported the proliferation of DG44 cells and reached a cell density of $2–3 \times 10^6$ cells per ml.

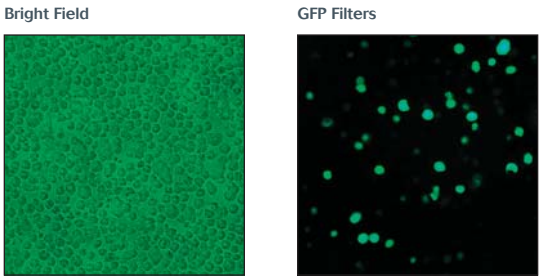
Table 1—Components of the DG44 Cells and Media Kit

Product	Quantity	Cat. no.
DG44 Cells	1.5×10^7 cells	12609-012
CD DG44 Medium	1000 ml	12610-010
Pluronic® F-68	100 ml	24040-032
L-Glutamine	100 ml	25030-081

Easily transfect with the DG44 transfection kit

The DG44 Transfection Kit includes all materials and a proven protocol for transfection of DG44 cells in suspension culture (Table 2). The optimized protocol uses the animal origin component-free Lipofectamine™ 2000 CD Transfection Reagent to transfect cells. This new protocol is simple to execute because lipid-mediated transfection requires fewer reagents, less time, and less effort compared to other transfection methods. It routinely yields 10-20% transfection efficiency (Figure 3). You can increase the efficiency by further optimization of the plasmid DNA and transfection lipid concentrations.

Figure 3—High-efficiency transfection of CHO-DG44 cells



Transfection of DG44 cells in suspension. DG44 cells were transfected with pTracer-GFP plasmid and Lipofectamine™ 2000 CD complexes. Photographs were taken in Bright and Fluorescence fields using GFP filters at 200X high power.

Table 2—Components of the DG44 Transfection Kit

Product	Cat. no.	Quantity
DG44 Cells	12609-012	1.5×10^7 cells
CD DG44 Medium	12610-010	1,000 ml
Pluronic® F-68	24040-032	100 ml
L-Glutamine	25030-081	100 ml
pTracer™-SV40 vector (GFP control)	V871-20	20 µg
Lipofectamine™ 2000 CD Transfection Reagent	12566-014	1 ml
OptiPro™ SFM	12309-019	1,000 ml

Boost production with growth optimization kits

To maximize protein production levels, you may need to optimize growth conditions to meet the needs of your specific applications. GIBCO™ offers two kits that allow you to do this in a convenient manner—the CD CHO Optimization Kit and Lipid Optimization Kit. The CD CHO Optimization Kit provides simplified, chemically defined basal media and selected supplements so you can easily test concentrations of various media components, such as Pluronic® F-68, glucose, sodium chloride, and iron chelates, and find the best combination for your experiment (Table 3). Recent reports suggest that use of cholesterol lipid concentrate can boost protein production in CHO and other mammalian cell lines. The Lipid Optimization Kit provides individual solutions that can be titrated and combined to optimize protein production conditions for a particular cell line (Table 4).

Table 3—Components of the CD CHO Optimization Kit

Product	Quantity
CD CHO Basal Media Part A	1 × 100 ml
CD CHO Basal Media Part B	2 × 100 ml
CD CHO Basal Media Part C	1 × 100 ml
Iron Chelate Kit	1 kit
10% Pluronic® F-68	1 × 100 ml
30% Sodium Chloride	1 × 100 ml
30% Glucose	1 × 100 ml

Table 4—Components of the Lipid Optimization Kit

Product	Quantity
Cholesterol Solution	1 × 20 ml
Fatty Acid Solution A	1 × 20 ml
Fatty Acid Solution B	1 × 20 ml
Fatty Acid Solution C	1 × 20 ml
Fatty Acid Solution A/B/C	1 × 20 ml

Ordering information

Product	Size	Cat. no.	Price
DG44 Cells and Media Kit (Industrial)	1 kit	12613-014	$270.00
CD DG44 Medium	1,000 ml	12610-010	$60.00
DG44 Transfection Kit	1 kit	12617-015	$950.00
CD CHO Optimization Kit	1 kit	004-0016SA	$704.83
Lipid Optimization Kit	1 kit	004-0030SA	$375.00

For further information on these or other GIBCO™ products, contact Technical Services:

United States Tech-Line℠: 1 800 955 6288

Canada Tech-Line℠: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO™ products catalog for the Tech-Line℠ in your region.

You may also contact your Invitrogen Sales Representative or visit **www.invitrogen.com**.

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