



293 SFM II

A defined, serum-free medium formulated without human or animal origin components. Developed for the suspension adaptation and high density culture of 293 cells.

Cat. No. 11686-029

Size: 1000mL

293 SFM II, 1X liquid, without L-glutamine

Storage Conditions: 2 to 8°C, in the dark.

Intended Use

293 SFM II is used to adapt and support the growth and expansion of human 293 cells and is intended for laboratory research use only.

Features

- A serum-free formulation designed to support the high-density culture of 293 cells in suspension. 293 SFM II is not recommended for adherent 293 cell culture.
- Contains no human or animal-origin products.
- 293 SFM II has been formulated without L-glutamine to avoid problems associated with L-glutamine degradation and to maximize shelf life.
- Supports 293 cellular production of adenovirus and/or glycosylated recombinant protein, comparable in many instances to levels produced in serum-supplemented classical media.
- Supports the large-scale, high density growth of 293 cells in bioreactors.
- Facilitates the adaptation of monolayer dependent 293 cells to suspension culture growth.
- **Low in protein content (10 mg/L).** The protein constituents are entirely of recombinant human forms.
- Supports the expansion of transfected 293 cells.
- Supports the high density suspension culture of HeLa S3 cells.
- In the hands of customers, 293 SFM II has been demonstrated to support the growth of PerC6 cells.

Introduction

The 293 cell line was produced by the transformation of human embryonic kidney cells by a sheared adenovirus type 5 DNA (1). The 293 cell line is important as a research tool due to its ability to produce adenovirus and glycosylated human recombinant protein(s).

293 SFM II is a very low protein, serum-free medium developed to support the adaptation of adherent-dependent 293 cells to high density suspension culture (see Adaptation Protocol). In addition to promoting adaptation to suspension conditions, 293 SFM II also supports the capability of 293 suspension cells to produce adenovirus or recombinant protein. 293 cells have traditionally been grown in two-dimensional monolayer cell cultures with some version of a serum-supplemented complex basal medium. Due to the inclination of 293 cells to aggregate, these cells have not routinely been grown in suspension. The availability of 293 SFM II represents an enabling technology allowing investigators to maximize experimental results with suspension cultures while eliminating concerns associated with the use of serum.

Cell Culture Procedures

General Information

Typically, 293 cells grown in 293 SFM II demonstrate a doubling time in the range of 26-40 hours. Under our conditions 293 cells cultured in 293 SFM II have typically achieved cell densities of $\sim 3 \times 10^5$ cells/mL in shaker or spinner culture (see Figure 1) and $\sim 4 \times 10^6$ cells/mL in bioreactor culture. Individual culturing and passaging techniques together with cellular heterogeneity inherent within the 293 cellular population may contribute to the demonstration of different results.

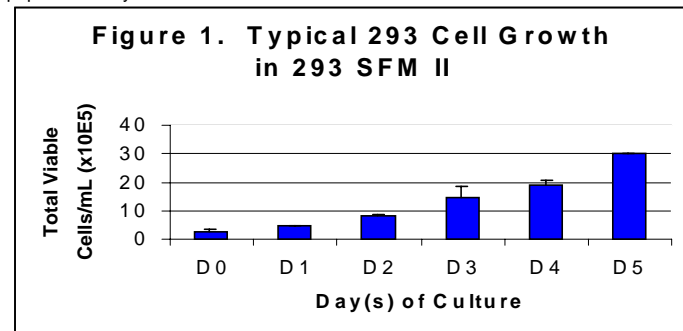


Figure 1. Typical 293 Cell Growth in 293 SFM II

Conditions: 125mL plastic shaker flasks, maximum fill volume 20mL, initial seeding density 3×10^5 cells/mL, 37°C in a humidified atmosphere of 8% CO₂ in air. Figure 1 represents the results of six experiments with each experimental data point conducted in triplicate.

293 SFM II requires supplementation with 4 mM L-glutamine (Cat. No. 25030) or GLUTAMAX™ I (Cat. No. 35050). Addition of a surfactant such as PLURONIC® F68 is not required.

Adaptation Protocol For Adapting 293 Cells From An Adherent-Dependent Culture Mode To Suspension Culture

1. If 293 cells are currently being cultured in a serum-supplemented medium, remove as much medium as possible. Displace 293 cells from the flask's surface by

rapping the flask sharply against your hand or a protected surface several times.

2. **Do not use trypsin or other proteolytic agents to dislodge cells.** Resuspend dislodged cells in 5 mL of 293 SFM II. Triturate with a small bore pipette until (most) cell clumps are dispersed into a single-cell suspension. Count the cells in the presence of 0.4% Trypan Blue solution (Cat. No. 15250 at a 1:9 (v/v) dilution of Trypan Blue solution to cell suspension) in a hemocytometer to determine cell concentration and viability. Automated methods such as the Coulter counter may also be used to determine cell density.
3. Dilute the cells with 293 SFM II (warmed to 37°C) to a density of 1.0×10^6 cells/mL. A total volume of 20 mL of the cell solution should be put into each disposable sterile 125 mL plastic Erlenmeyer flask as determined necessary.
4. Incubate the shake flask(s) on a rotary shaker at 125 rpm in a humidified 37°C incubator (gassed with 8% CO₂ in air). *Cells will not thrive in 293 SFM II at 5% CO₂ levels.*
5. Determine cell count and viabilities daily. When the viable cell count reaches $\sim 1.5 \times 10^5$ cells/mL, dilute to $2.5\text{-}3.0 \times 10^5$ cells/mL with warmed (37°C) 293 SFM II. Continue to dilute to $2.5\text{-}3.0 \times 10^5$ cells/mL whenever the cell densities are $\geq 7.5\text{-}10 \times 10^5$ cells/mL.
6. After adaptation to growth in serum-free suspension culture, it is possible to scale-up the cultures in spinner flasks or bioreactors. The appropriate spinner or impeller speed should be individually determined. Under our conditions, the optimum spinner speed was ~ 150 rpm for a simple T-shaped impeller and 70-100 rpm impeller speed in our bioreactor system configuration.

Caution: Some spinner apparatus emit significant heat and water-jacketed incubators usually cannot readily equilibrate to temperature variations. Temperatures > 40°C are lethal to 293 cells.

Note: Invitrogen now offers 293-F (Cat. No. 11625) and 293-H (Cat. No. 11631) cells which have been pre-adapted to growth in 293 SFM II.

Transfection Protocol

Note: Complex formation of DNA with transfection reagents such as LIPOFECTAMINE Plus™ and LIPOFECTAMINE™ 2000 are inhibited by constituents of 293 SFM and 293 SFM II. Therefore, these transfection reagents and media should not be used together.

Prepare DNA-liposome complexes:

1. To each well of a six-well plate, add 1 mL OPTI-MEM® I Reduced Serum Medium (Cat. No. 31985).
2. For each transfection, add 3-4 µg of DNA to each well. Gently swirl to mix.
3. For each transfection, add 10-20 µL of LIPOFECTAMINE™ 2000 to each well. Gently swirl the plate repeatedly to mix the DNA and lipid.
4. Incubate at room temperature for 20-45 minutes to allow DNA-liposome complexes to form. Although the solution may appear somewhat cloudy it will not impede the process of transfection.

While the DNA-liposome complexes are forming, prepare the 293 cells for transfection:

5. Determine the viable cell count of a suspension culture of 293 cells growing in 293 SFM II (Cat. No. 11686). Cells must be in logarithmic growth prior to transfection for optimal results.
6. Calculate the volume of cell suspension required to plate 293 cells into six-well plates at 2×10^6 cells/well. Include two wells (4×10^6 cells) overage. Transfer this volume of cell suspension to a sterile 50 mL centrifuge tube. Bring the total volume to 50 mL with OPTI-MEM I.
7. Pellet cells by centrifuging the tube at 100 x g for 4 minutes.
8. Gently aspirate the supernatant, being sure not to disturb the cell pellet.
9. Resuspend the cell pellet using 10 mL of OPTI-MEM I, triturating to achieve a single cell suspension. Bring the total volume to 50 mL with OPTI-MEM I.
10. Pellet cells by centrifuging the tube at 100 x g for 4 minutes. This second wash step is imperative for optimal transfection efficiency.
11. Gently aspirate the supernatant, being careful not to disturb the cell pellet.
12. Resuspend the cell pellet using 200 µL of OPTI-MEM I for every 2×10^6 cells contained in the tube. This will yield a solution of 1×10^7 cells/mL.
13. Add 200 µL of cell suspension to each well of the six-well plate containing the DNA-liposome complexes. Pipet up and down to mix well.
14. Incubate the plate for 5 hours at 37°C in a 8% CO₂ incubator. There is no need to remove the transfection mixture, nor to feed with growth medium.
15. For **transient expression**, harvest and assay cell extracts or stain cells *in situ* for reporter gene activity at 24 hours after the start of transfection. For **stable expression**, passage the cells at 24 hours post transfection using the same seeding density or split ratio that is normally used. At 48 hours post transfection, replace the medium on the cells with medium containing the appropriate selective antibiotic (e.g. GENETICIN®). Note: Suspension cells will need to be pelleted by centrifugation (100 x g for 4 minutes) before receiving medium containing selective antibiotic.

Note: When using different sized tissue culture plates, adjust the amount of DNA, LIPOFECTAMINE™ 2000 and cell concentration in proportion to the difference in surface area of the vessel.

293 cells cultured in 293 SFM II may grow as 2-10 cell clusters, therefore samples should be vortexed vigorously before passaging or counting. Optimal vortexing conditions must be determined by the user based upon speed, time and viability.

Customers wishing to transfect 293 cells in an anchorage-dependent system using serum-supplemented medium, are urged to call the Invitrogen Tech-Line™ for an established protocol at the number listed below.

References:

1. Graham, F. L., Smiley, J., Russell, W. C. and R. Nairn (1977) *J. Gen. Virol.* 36, 59.

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For further information on this or other GIBCO™ products, contact Technical Services at the following:

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 catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

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February 2003

Form No. 3919