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life technologies

# 293 SFM II (1X)

## Description

293 SFM II is a serum-free, human, and animal-origin free, low-protein (<10 µg/mL) formulation optimized for high-density suspension culture of 293 (human embryonic kidney) cells. 293 SFM II supports HEK 293 cellular production of adenovirus and/or glycosylated recombinant protein, comparable to levels produced in serum-supplemented media. 293 SFM II is not recommended for adherent 293 cell cultures. 293 SFM II has been demonstrated to support the growth of Per.C6® cells and the high-density suspension culture of HeLa S3 cells.

| Product | Catalog no. | Amount | Storage | Shelf life* |
|---|---|---|---|---|
| 293 SFM II (1X), liquid | 11686-029 | 1000 mL | 2°C to 8°C; Protect from light | 6 months |

* Shelf life duration is determined from Date of Manufacture.

## Product use

For Research Use Only. Not for use in diagnostic procedures.

## Important information

293 SFM II facilitates the adaptation of monolayer dependent 293 cells to growth in suspension culture.

## Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Prepare medium

- 293 SFM II Medium requires aseptic supplementation with 4 mM L-glutamine or GlutaMAX™-I prior to use.
- Antibiotics are not recommended; however, 5 mL/L of Penicillin-Streptomycin (100X) may be used when required.
- Addition of a surfactant such as Pluronic® F-68 is not required.

## Culture conditions

**Media:** Complete 293 SFM II Medium.

**Cell line:** HEK 293 cells.

**Culture type:** Suspension

**Culture vessels:** Shake flask, spinner bottle, or bioreactor.

**Temperature range:** 36°C to 38°C.

**Incubator atmosphere:** Humidified atmosphere of 8% $CO_2$ in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Adapt to suspension culture in 293 SFM II

**Note:** We offer 293-F and 293-H cells that have been pre-adapted to growth in 293 SFM II.

1. Aspirate media from cell monolayer and displace 293 cells from the flask surface by rapping the flask sharply against your hand or a protected surface several times.

   **Note:** Do not use trypsin or other proteolytic agents to dislodge cells.

2. Resuspend dislodged cells in 5 mL of 293 SFM II.

   **Note:** 293 cells cultured in 293 SFM II may grow as clusters of 2–10 cells.

3. Disperse clusters into a single-cell suspension by triturating with a small bore pipette or vortexing before passaging or counting. Optimal vortexing conditions must be determined based upon speed and duration versus viability.

4. Determine viable cell density using a Countess® Automated Cell Counter. Alternate methods (e.g., Coulter counter or hemocytometer) may also be used.

5. Dilute cells in prewarmed complete 293 SFM II to a viable cell density of $1 \times 10^6$ cells/mL. Dispense cell suspension, up to a maximum of 30 mL/flask, into sterile 125-mL Erlenmeyer shake flasks.

6. Incubate the shake flask(s) on a rotary shaker (125–130 rpm) at 37°C in a humidified atmosphere of 8% $CO_2$ in air.

   **Note:** Cells will not thrive in 293 SFM II at lower $CO_2$ levels (e.g., 5% $CO_2$).

7. When the viable cell density reaches ~$1.5 \times 10^6$ cells/mL, dilute the cells with prewarmed, complete 293 SFM II to $2.5 \times 10^5$–$3.0 \times 10^5$ cells/mL.

8. After the first passage, dilute cells to $2.5 \times 10^5$–$3.0 \times 10^5$ cells/mL whenever the viable cell density exceeds $0.75 \times 10^6$–$1 \times 10^6$ cells/mL. After several passages of consistent growth and viability in 293 SFM II, the culture is considered to be adapted.

**Note:** 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.

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

## Transfection protocol

**Important:** Complex formation of DNA with transfection reagents such as Lipofectamine® Plus and Lipofectamine® 2000 are inhibited by constituents of 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® Reduced Serum Medium.
2. For each transfection, add 3–4 µg of DNA to each well. Gently swirl the plate to mix.
3. For each transfection, add 10–20 µL of Lipofectamine® 2000 transfection reagent to each well. Gently swirl to mix the DNA and lipid.
4. Incubate at room temperature for 20–45 minutes, and repeatedly gently swirl the plate allowing DNA-liposome complexes to form. Although the solution may appear somewhat cloudy it will not impair transfection.

### Prepare 293 cells for transfection:

1. Determine the viable cell density of a suspension culture of 293 cells growing in 293 SFM II.

   **Note:** Cells should be in mid-log phase growth prior to transfection for optimal results.
2. Calculate the volume of cell suspension required to plate 293 cells into six-well plates at $2 \times 10^6$ cells/well. Include two wells ($4 \times 10^6$ 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® Reduced Serum Medium.
3. Centrifuge cell suspension at 200 × *g* for 5 minutes. Aspirate and discard the supernatant, being sure not to disturb the cell pellet.
4. Resuspend the cell pellet in 10 mL Opti-MEM® Reduced Serum Medium, pipetting up and down to achieve a single-cell suspension. Add Opti-MEM® Reduced Serum Medium to a total volume of 50 mL.
5. Centrifuge cell suspension at 200 × *g* for 5 minutes. Aspirate and discard the supernatant, being sure not to disturb the cell pellet.

   **Important:** This second wash step is imperative for optimal transfection efficiency.
6. Resuspend the cell pellet in 200 µL of Opti-MEM® Reduced Serum Medium for every $2 \times 10^6$ cells contained in the tube. This will yield a suspension of $1 \times 10^7$ cells/mL.
7. Add 200 µL of cell suspension to each well of the six-well plate containing DNA-liposome complexes. Pipet up and down to mix well.
8. Incubate at 37°C for 5 hours in a humidified 8% $CO_2$ incubator. There is no need to remove the transfection mixture, or to feed with growth medium.

**Note:** Transfection procedure can be scaled up or down by adjusting the amount of DNA, Lipofectamine® 2000 transfection reagent and cell concentration in proportion to the difference in surface area of the culture vessel.

### Transient expression

Harvest and assay cell extracts or stain cells *in situ* for reporter gene activity at 24 hours after the start of transfection.

### Stable expression

1. Passage the cells at 24 hours post-transfection using the same seeding density or split ratio that is normally used.
2. At 48 hours post-transfection, replace spent medium with medium containing the appropriate selective antibiotic (e.g., Geneticin®).

   **Note:** Suspension cells will need to be collected by centrifugation (100 × *g* for 4 minutes) before changing spent medium for medium containing selective antibiotic.

## Related products

| Product | Catalog no. |
|---|---|
| L–Glutamine-200mM (100X), Liquid | 25030 |
| GlutaMAX™-I, 200mM (100X), Liquid | 35050 |
| 293 F Cells, SFM Adapted | 11625 |
| 293 H Cells, SFM Adapted | 11631 |
| Geneticin® (G-418 Sulfate) | 11811 |
| Opti-MEM® I Reduced Serum Medium (1X), Liquid | 31985 |
| Penicillin-Streptomycin 100X Solution | 15070 |
| 293Fectin™ Transfection Reagent | 12347 |
| Lipofectamine® 2000 Transfection Reagent | 11668 |
| Trypan Blue Stain | 15250 |
| Countess® Automated Cell Counter | C10227 |

## Explanation of symbols and warnings

The symbols present on the product label are explained below:

| | | | | |
|---|---|---|---|---|
|  |  |  |  |  |
| Temperature Limitation | Manufacturer | Batch code | Use By: | Catalog number |
|  |  |  |  | |
| Caution, consult accompanying documents | Consult instructions for use | Keep away from light | Sterilized using aseptic processing techniques | |

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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For further assistance, email **techsupport@lifetech.com**.



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