gibco
by Thermo Fisher Scientific

293 Cells, SFM Adapted

Description

293-F and 293-H Cells are clonal isolates selected for their superior serum-free growth and transfection efficiency derived from adenovirus type 5 transformed embryonal human kidney cells expressing the E1A adenovirus gene. The 293-F strain is a fast-growing variant of the 293 cell line. The 293-H strain is a variant, which when grown in serum supplemented medium, demonstrates better adherence in monolayer culture and ease of use for plaque assays and other anchorage dependent applications. Both 293-F and 293-H cell lines can be adapted to serum-free suspension culture in 293 SFM II or CD 293 Medium supplemented with L-glutamine for recombinant protein production and adenovirus propagation. 293-F and 293-H cells as provided are adapted to CD 293 Medium.

Product	Catalog no.	Amount	Storage
293-F Cells, SFM Adapted	11625-019	1 vial*	–200°C to –125°C, Liquid nitrogen
293-H Cells, SFM Adapted	11631-017	1 vial*	–200°C to –125°C, Liquid nitrogen

* 1 vial contains ≥7.5 × 10^6cells/vial

Safety information

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

Caution: Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HB_sAg. Handle in accordance with established bio-safety practices.

Important information

Cells are stable when maintained at –200°C to –125°C.

Prepare medium

CD 293 Medium and 293 SFM II require aseptic supplementation with L-glutamine or GlutaMAX™-I prior to use.

Add 20 mL/L GlutaMAX™-I (200mM) or L-glutamine, 4 mM final concentration, to the medium before use.

Note: Antibiotics are not recommended; however, 5 mL/L of Penicillin-Streptomycin (100X solution) may be used when required.

Culture conditions

Media: Complete 293 SFM II or CD 293 Medium, supplemented with 4 mM GlutaMAX™-I or L-glutamine.

Cell line: 293 human embryonic kidney cells.

Culture type: *Stationary*- adherent monolayer; or *Suspension*- orbital shaker platform rotating at 120–130 rpm.

Culture vessels: T-flasks or shake flasks.

Temperature range: 36°C to 38°C.

Incubator atmosphere: Humidified atmosphere of 7–9% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a 125 mL shake flask containing 30 mL pre-warmed complete 293 SFM II or CD 293 Medium for suspension culture.
3. Incubate at 37°C in a humidified atmosphere of 7–9% CO_2 in air. Loosen flask caps to allow for gas exchange.
4. Subculture cells 3–5 days post thaw when viable cell density reaches 2 × 10^6–3 × 10^6 viable cells/mL. Cell viability should be ≥90% and growth rate in mid-logarithmic phase.

Note: We recommend subculturing cells for a minimum of 3 passages before use in other applications or transfer into serum-supplemented media.

Subculture cells

293 cells may be subcultured either in suspension culture or as adherent monolayers depending upon the application.

1. Subculture cells 3–5 days post thaw or when cultures reach 60–80% confluence (adherent monolayer), or a viable cell density of 2 × 10^6–3× 10^6 viable cells/mL (suspension). Ensure that cell viability is ≥90% and growth rate is in mid-logarithmic phase. For suspension culture proceed to step 3.
2. Displace cells from the flask surface by rapping sharply against your hand.
3. Transfer cell suspension to a sterile conical tube. Ensure cells are dispersed in a monocellular suspension by gently pipetting up and down or vortexing for up to 40 seconds.
4. Determine total viable cell density using a Countess™ Automated Cell Counter (alternate automated or manual methods may be used).
5. Seed cells into fresh prewarmed complete medium at the appropriate density for your chosen culture method.
   a. Adherent: 2 × 10^4–5 × 10^4 viable cells/cm^2 in 2–3 mL fresh complete pre-warmed medium per 10 cm^2.
   b. Suspension: 3 × 10^5 viable cells/mL fresh complete pre-warmed medium.

Note: 293 suspension cultures may grow as 2–10 cell clusters. Vigorous vortexing for up to 40 seconds may be required at each subculture for a number of passages until the cultures grow as a single cell suspension.

Scaling-up 293 cell cultures

You may scale up the 293 cultures in spinner flasks or bioreactors using the following guidelines.

- We recommend using 293 SFM II for high-density suspension culture of 293 cells.
- Determine optimal seeding density for each system. We recommend seeding densities of 3 × 10^5–5 × 10^5 viable cells/mL.

Note: If the split ratio of cells to fresh media is <1:2, centrifuge cell suspension at 200 × *g* for 5 minutes and resuspend the cell pellet in fresh 293 SFM II prior to inoculating the spinner or bioreactor culture.

- Optimize the spinner or impeller speed for your bioreactor depending on your needs.
- Higher stirring speeds and/or impeller design may necessitate supplementation of 293 SFM II or CD 293 Medium with 2.5–5 mL/L Pluronic™ F-68 (10%) to avoid sheer stress in the culture.

Cryopreservation

1. Prepare the desired quantity of 293 cells in suspension culture, harvesting in mid-log phase of growth, viable cell density of 0.5×10^6–1×10^6 viable cells/mL, and viability >90%. Retain the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of $\geq 5 \times 10^6$ cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% growth media (50:50 ratio of fresh to conditioned media) +7.5% DMSO and store at 4°C until use.

   IMPORTANT! Prepare cryopreservation medium on the day of intended use.
4. Harvest cells by centrifugation at 200 × *g* for 5 minutes. Discard the supernatant and resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1 mL in a 2-mL cryovial).
6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase); we recommend storage at –200°C to –125°C.

Transfection

Several days prior to transfection, we recommend transferring cells to adherent (monolayer) culture in serum-supplemented media (i.e., Dulbecco's Modified Eagle Medium supplemented with 0.1 mM MEM Non-Essential Amino Acids and 10% Fetal Bovine Serum). **Do not** add antibiotics to the media during transfection. Cells should adapt directly into the serum-supplemented media without any trouble. After transfection and selection, 293-F or 293-H cells can be expanded and re-adapted back into serum-free suspension culture in 293 SFM II or CD 293 Medium supplemented with 4 mM L-glutamine or GlutaMAX™-I and the appropriate selective antibiotic. For optimal transfection results, we recommend using Lipofectamine™ 2000 or 293Fectin™ Transfection Reagent. Refer to the accompanying product manuals for instructions. Other transfection reagents may be used if desired.

Note: CD 293 and 293 SFM II can inhibit complex formation of DNA with some cationic lipid transfection reagents (e.g., Lipofectamine™ 2000, 293Fectin™, Lipofectamine™, and Lipofectin™). If you are using one of these transfection reagents, culture cells in another medium immediately prior to and during the transfection.

Related products

Product	Catalog no.
293 SFM II	11686
CD 293 Medium	11913
L-Glutamine-200 mM (100X), Liquid	25030
GlutaMAX™	35050
Pluronic™ F-68, 10% (100X)	24040
Penicillin-Streptomycin, Liquid	15070
Gentamicin	15750
DMEM, high glucose	11965
MEM Non-Essential Amino Acids (100X), Liquid	11140
Qualified FBS, US	26140
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:

		
Manufacturer	Catalog number	Batch code
		
Caution, consult accompanying documents	Consult instructions for use	Temperature Limitation

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**.

LIMITED USE LABEL LICENSE No. 561: Internal Research and Development Use of Mammalian and Insect Cell Lines

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product and progeny, to perform internal research and development for the sole benefit of the purchaser. The purchase of this product does not grant the purchaser any additional rights, including, without limitation: (a) the right to use the product or its components in commercial applications of any kind, including bioproduction (e.g. use of the product to manufacture therapeutic agents or diagnostic test components, such as proteins, antibodies, viral particles or virus-like particles), quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration; (b) the right to use the product or its components as a therapeutic agent or diagnostic test component; (c) the right to use the product or its components to produce material for use in human clinical trials; or (d) the right to transfer or resell the product or its components in any form, progeny or derivative. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit **www.thermofisher.com/support**.
For further assistance, email **techsupport@lifetech.com**.