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FreeStyle™ 293 Expression Medium

Description

FreeStyle™ 293 Expression Medium is a chemically-defined, protein-free medium specifically developed for the ability to support the growth and transfection of FreeStyle™ 293-F cells under suspension type culture conditions. FreeStyle™ 293 Expression Medium is a complete, ready-to-use medium that has been supplemented with GlutaMAX™-I Supplement and is animal-origin free.

Product	Catalog No.	Amount	Storage	Shelf Life*
FreeStyle™ 293 Expression Medium	12338-018 12338-026 12338-001 12338-002	1000 mL 6 × 1000 mL 10 L, bag 20 L, bag	2°C to 8°C; Protect from light	12 months

* Shelf Life duration is determined from Date of Manufacture.

Product Use

Caution: For manufacturing, processing, or repacking.

Safety Information

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

Prepare Media

- FreeStyle™ 293 Expression Medium contains GlutaMAX™-I supplement and does not require further supplementation with L-glutamine or GlutaMAX™-I supplement.
- Antibiotics are not recommended; however, 5 mL/L of Antibiotic-Antimycotic or Penicillin-Streptomycin may be used when required.

Culture Conditions

Media: FreeStyle™ 293 Expression Medium

Cell Line: FreeStyle™ 293-F Cells, 293-F Cells

Culture Type: Suspension

Culture Vessels: 125-mL or 250-mL Shake Flask

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.

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 prewarmed FreeStyle™ 293 Expression Medium.
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125–135 rpm. Loosen flask caps to allow for gas exchange.
4. Subculture cells 3–5 days post thaw. Ensure that the viable cell density is 1×10^6 cells/mL, viability is ≥90%, and growth rate is in mid-logarithmic phase prior to subculturing. If cell density does not reach 1×10^6 viable cells/mL within 5 days, centrifuge cells at 200 × *g* for 5 minutes and resuspend cell pellet in 20–30 mL of fresh FreeStyle™ 293 Expression medium.

Subculture Cells

Subculture 293-F cells directly into FreeStyle™ 293 Expression Medium.

1. Determine viable cell density using a Countess® Automated Cell Counter.
2. After first passage allow cell density to reach 2–3 × 10^6 viable cells/mL prior to dilution.
3. For optimal performance and cell growth dilute cells at a seeding density of 3 × 10^5 viable cells/mL every 3–4 days with fresh medium.
4. It is recommended to subculture cells for a minimum of two passages before use.

Note: FreeStyle™ 293-F cultures may grow as 2–10 cell clusters. Prior to passage of cells into fresh medium, allow the culture to sit briefly to allow large cell clumps to settle to the bottom of the culture vessel, then carefully remove the remaining suspended cells into new vessels containing fresh medium. Vigorous vortexing for 10–30 seconds may be required at each subculture for a number of passages until the cultures grow predominantly as single cells. If visible clumping of cells in FreeStyle™ 293 Expression Medium is observed, addition of 1 mL/L of Anti-Clumping Agent can be added. However, if the cells will be used for transfection, culture medium should not contain Anti-Clumping Agent.

Transfection

Prepare Cells for Transfection

Anti-Clumping Agent is incompatible with all cationic transfection reagents. Clumping of cells lowers transfection efficiency, to minimize clumping in cultures used for transfection, it is recommended to:

- Remove Anti-Clumping Agent 1–3 passages prior to transfection.
- Maintain the cells at a low density, between 0.2–2 × 10^6 viable cells/mL.
- Increase agitation rate of the shaker or spinner platform, but without decreasing cell viability below 95%.
- Passage the cells frequently (e.g., 3X per week).

High efficiency transfection of FreeStyle™ 293-F cells grown in FreeStyle™ 293 Expression medium can be achieved using either FreeStyle™ MAX or 293fectin™ transfection reagents. For protocol details, please refer to the respective transfection reagent manuals, or refer to our website: **www.lifetechnologies.com**.

General Transfection Considerations

1. Cells are typically transfected at a density of 1×10^6 cells/mL.
2. Dilute cells one day prior to transfection in fresh FreeStyle™ 293 Expression Medium so that they will reach a density of $1.2–1.5 \times 10^6$ cells/mL on the day of transfection.
3. Adjust cell density to 1×10^6 cells/mL with fresh, pre-warmed FreeStyle™ 293 Expression Medium immediately prior to transfection.
4. No medium exchange or addition of fresh FreeStyle™ 293 Expression Medium is required post-transfection. The cells should maintain >50% viability for 5–7 days post-transfection.

Cryopreservation

1. Prepare the desired quantity of cells, harvesting when the viable cell density reaches $0.5–2 \times 10^6$ cells/mL (mid-log phase) with viability >90%. Reserve 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 $0.5–1 \times 10^7$ cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% FreeStyle™ 293 Expression Medium (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 $200 \times g$ for 5–10 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Immediately dispense aliquots of this cell 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) storage at –200°C to –125°C is recommended.

Related Products

Product	Catalog No.
FreeStyle™ 293-F Cells	R790-07
FreeStyle™ 293 Expression System	K9000-01
293-F Cells, SFM Adapted	11625
293fectin™ Transfection Reagent	12347
FreeStyle™ MAX Reagent	16447
FreeStyle™ MAX 293 Expression System	K9000-10
Opti-MEM® I Reduced Serum Medium (1X), liquid	31985
OptiPRO™ SFM (1X), liquid	12309
Pluronic® F-68, 10% (100X)	24040
Antibiotic-Antimycotic (100X), liquid	15240-112
Penicillin-Streptomycin, liquid	15140
Anti-Clumping Agent	0010057
Countess® Automated Cell Counter	C10227
Trypan Blue Stain	15250

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

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

Limited Use Label License: Internal Research and Bioproduction Use

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) to culture cells for the purpose of producing a product wherein the product will be used for any or all of the following: (i) internal research use by the purchaser; (ii) resale for internal research use by third parties; (iii) performance of research conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties; (iv) resale for use as a human therapeutic agent or diagnostics product or component by third parties; (v) performance of manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties.

The purchase of this product does not grant the purchaser any additional rights, including (without limitation) the right to transfer or resell the product in any form, the right to use the product as a therapeutic agent or diagnostics test component, or to use the product to perform other tests on a contract or fee per test basis for or on behalf of third parties. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Product Warranty

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