

# FreeStyle™ F17 Expression Medium

## Description

FreeStyle™ F17 Expression Medium is a chemically-defined, serum-free and animal origin-free medium containing no proteins, hydrolysates, or components of undefined composition. FreeStyle™ F17 Expression Medium was developed for the ability to support the growth and transfection of FreeStyle™ 293-F cells under suspension-type culture conditions. FreeStyle™ F17 Expression Medium also supports the growth and transfection of suspension Chinese Hamster Ovary (CHO) cells. FreeStyle™ F17 is a multi-platform medium compatible with a range of transient expression systems and may be used as an alternative to FreeStyle™ 293 or FreeStyle™ CHO Expression Media.

Product	Catalog no.	Amount	Storage	Shelf life*
FreeStyle™ F17 Expression Medium	A13835-01	1000 mL	2°C to 8°C; Protect from light	12 months
	A13835-02	6 × 1000 mL		
	A13835-03	10 L bag		
	A13835-04	20 L bag		

\*Shelf Life duration is determined from Date of Manufacture.

## Product use

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

## Safety information

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

## Prepare media

FreeStyle™ F17 Expression Medium requires supplementation with L-glutamine or GlutaMAX™-I supplement. The addition of a surfactant such as Pluronic® F-68 is not required (see **Large scale culture**).

1. Add 200 mM L-glutamine or GlutaMAX™-I to 8 mM final concentration (40 mL/L), to the medium before use.
2. Antibiotics are not recommended; however, 5 mL/L of Antibiotic-Antimycotic or Penicillin-Streptomycin may be used when required.

## Culture conditions

**Media:** FreeStyle™ F17 Expression Medium

**Cell line:** FreeStyle™ 293-F Cells (recommended), 293-F Cells, CHO 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<sub>2</sub> in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Recovery

1. Rapidly thaw (<1 minute) frozen cryovial of FreeStyle™ 293-F 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 complete FreeStyle™ F17 Expression Medium.
3. Incubate at 37°C in a humidified atmosphere of 8% CO<sub>2</sub> 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.

## Subculture cells

Subculture FreeStyle™ 293-F cells directly into FreeStyle™ F17 Expression Medium.

1. Determine viable cell density using a Countess® Automated Cell Counter. Alternate methods (e.g. Coulter counter or hemocytometer) may also be used.
2. Ensure that the viable cell density is  $\geq 1 \times 10^6$  cells/mL, viability is  $\geq 90\%$ , and growth rate is in mid-logarithmic phase prior to subculturing. If cell density does not reach  $1 \times 10^6$  viable cells/mL within 5 days, centrifuge cells at  $100 \times g$  for 5 minutes and resuspend cell pellet in 20–30 mL of fresh FreeStyle™ F17 Expression medium.
3. After first passage allow cell density to reach  $2\text{--}3 \times 10^6$  viable cells/mL prior to dilution.
4. For optimal performance and cell growth, dilute cells to a seeding density of  $3 \times 10^5$  viable cells/mL every 3–4 days with fresh medium.
5. We recommend subculturing cells 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™ F17 Expression Medium is observed, you can add 1 mL/L of Anti-Clumping Agent. However, if the cells will be used for transfection, culture medium should not contain Anti-Clumping Agent.

## Adapt cells to FreeStyle™ F17 Expression Medium

Most 293 or CHO cell lines will adapt directly from conventional serum-containing or other serum-free medium. It is critical that cell viability be at least 90% and cells be in the mid-logarithmic phase of growth prior to adaptation. After several passages in 100% FreeStyle™ F17 Expression Medium, the viable cell count should reach  $2\text{--}3 \times 10^6$  cells/mL with a viability exceeding 85% within 4–6 days of culture. At this stage the culture is considered to be adapted to FreeStyle™ F17 Expression Medium.

## 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, we recommend to:

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

High efficiency transfection of FreeStyle™ 293-F cells grown in FreeStyle™ F17 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 visit: [www.lifetechnologies.com](http://www.lifetechnologies.com).

### General transfection considerations

- Cells are typically transfected at a density of  $1 \times 10^6$  cells/mL.
- Dilute cells one day prior to transfection in fresh FreeStyle™ F17 Expression Medium so that they will reach a density of  $1.2\text{--}1.5 \times 10^6$  cells/mL on the day of transfection.
- Adjust cell density to  $1 \times 10^6$  cells/mL with fresh, pre-warmed FreeStyle™ F17 Expression Medium immediately prior to transfection.
- No medium exchange or addition of fresh FreeStyle™ F17 Expression Medium is required post-transfection. The cells should maintain >50% viability for 5–7 days post-transfection.

### Large scale culture

FreeStyle™ 293-F cultures can be scaled-up in spinner flasks or bioreactors. Optimize the appropriate spinner or impeller speed and seeding density for each culture system.

If the split ratio of cells to fresh media is less than 1:2, we recommend spinning down the cell suspension and resuspending the cell pellet in fresh, pre-warmed FreeStyle™ F17 Expression Medium prior to inoculating the spinner or bioreactor culture. Monitor cell viability and the degree of cell clumping. Extensive cell clumping may reduce transfection efficiency.

At high stirring speeds (i.e., greater than 130 rpm) and/or depending on impeller design, FreeStyle™ F17 Expression Medium may require supplementation with additional Pluronic® F-68 to avoid shear stress in the culture. Pluronic® F-68 concentration may need titration to determine the optimal level.

### Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of  $\geq 1 \times 10^7$  cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% FreeStyle™ F17 Expression Medium (50:50 ratio of fresh to conditioned media) + 7.5% DMSO. Store at 4°C until use.  
**IMPORTANT!** Prepare cryopreservation medium the day of use.
4. Harvest cells by centrifugation at  $100 \times g$  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.
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; we recommend vapor phase storage at  $-200^\circ\text{C}$  to  $-125^\circ\text{C}$ .

### 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
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:

				
Catalog number	Manufacturer	Use by	Keep away from light	Batch code
				
Caution, consult accompanying documents	Consult instructions for use	Temperature limitation	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.

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