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by Thermo Fisher Scientific

Expi293F™ Cells

Cat. nos.	Size	Conc. 1×10^7 cells/vial
A14527	1 vial	Store in liquid nitrogen
A14528	6 × 1 vial	

Pub. Part no. 100014707 **Pub. no.** MAN0006283 **Rev.** A.0

Description

The Expi293F™ cell line is a component of the Expi293™ Expression System. They are a variant of the 293 cell line that has been adapted to high-density suspension growth in Expi293™ Expression Medium. The 293 cell line is a permanent line established from primary embryonic human kidney transformed with sheared human adenovirus type 5 DNA (1,2). The E1A adenovirus gene expressed in 293 cells participates in transactivation of some viral promoters, allowing these cells to produce very high levels of protein.

The Expi293F™ cell line exhibits the following characteristics:

- Prepared from Master Cell Bank cultures derived from parental 293-F cells that were selected for high transfection efficiency and protein expression.
- Adapted to high density, serum-free, suspension growth in Expi293™ Expression Medium.
- Demonstrates high transfection efficiencies with ExpiFectamine™ 293 Reagent.
- Suspension cultures may be transfected in Expi293™ Expression Medium without any requirement for media change.
- Designed for scalable transient transfection and protein expression at culture volumes ranging from less than 1 mL (i.e., multi-well plates) to tens of liters.

As with other human cell lines, when working with Expi293F™ Cells, handle as potentially biohazardous material under at least Biosafety Level-2 (BL-2) containment. For more information on BL-2 guidelines, refer to *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed., published by the Centers for Disease Control, which is available for downloading at: **www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm**.

Preparing Expi293™ Expression Medium

Expi293™ Expression Medium (available separately, Cat. no. A14351) is a complete, serum-free, protein-free, and chemically defined medium that does not require any supplementation. Pre-warm the medium to 37°C prior to use.

Thawing Expi293F™ Cells

1. Remove the vial of cells from liquid nitrogen and place it in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly with gentle agitation. Do **not** submerge the vial in the water.
2. Just before the cells are completely thawed, decontaminate the vial by wiping it with 70% ethanol before opening it in a Class II biological safety cabinet.
3. Transfer the entire contents of the cryovial into a 125-mL polycarbonate, disposable, sterile Erlenmeyer shaker flask containing 29 mL of pre-warmed Expi293™ Expression Medium.
4. Incubate the cells in a 37°C incubator with humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125 rpm.

   Note: If not using a vent-cap flask, loosen the cap of the flask a quarter turn from snug to allow oxygenation/aeration of the culture.
5. Once the culture has reached >1 × 10^6 viable cells/mL (typically 2–4 days), aseptically take a sample of the cell suspension, and determine viable and total cell counts.
6. Subculture the Expi293F™ Cells by seeding shaker flasks at 3 × 10^5 viable cells/mL in pre-warmed Expi293™ Expression Medium. We generally use a 125-mL or 250-mL polycarbonate, disposable, sterile, Erlenmeyer flasks containing 30 mL or 60 mL total working volume of cell suspension, respectively.

Guidelines for passaging Expi293F™ Cells

- Subculture the Expi293F™ Cells a minimum of three times to allow them to recover from thawing before using them in transfection experiments.
- Subculture Expi293F™ Cells when they reach a density of approximately 3×10^6–5×10^6 viable cells/mL, typically every 3–4 days.
- Split the Expi293F™ culture to 0.3×10^6–0.5×10^6 cells.
- When maintaining Expi293F™ Cells, we generally use a 125-mL or 250-mL polycarbonate, disposable, sterile Erlenmeyer flask containing 25–40 mL or 50–80 mL total working volume of cell suspension, respectively.
- Glass flasks without baffles may be used, but thorough cleaning after each use is essential to avoid potential toxicity, which is more problematic in serum-free cultures.

Passaging Expi293F™ Cells

1. Aseptically take a small aliquot of the cell suspension and determine the viable and total cell counts manually using a hemocytometer or electronically using an automated cell counter.
2. Using the cell density determined in Step 1, calculate the split ratio needed to seed the new shaker flask at 0.3×10^6–0.5×10^6 viable cells/mL.
3. Dilute the cells in fresh, pre-warmed Expi293™ Expression Medium to give a final cell density of 0.3×10^6–0.5×10^6 viable cells/mL in the desired final volume.
4. Incubate flasks in a 37°C incubator containing a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125 rpm.
5. Repeat Steps 1–4 as necessary to maintain or expand cells.

References

1. Graham, F.L., *et al*. (1977) *J. Gen. Virol. 36, 59–74.*
2. Harrison, T., *et al*. (1977) *Virology 77, 319–329*.

Technical support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at **www.lifetechnologies.com**. For further assistance, email our Technical Support team at **techsupport@lifetech.com**.

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