Expi293F™ cell lines

For use with Expi293F™ cells, Expi293F™ GnTI- Cells, Expi293F™ Inducible Cells, and Expi293F™ Inducible GnTI- Cells

Catalog Numbers A14527, A14528, A39240, A39241, and A39242

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Expi293F™ Cells

Expi293F™ Cells are human cells derived from the 293F cell line, and are a core component of the Expi293™ Expression System. They are maintained in suspension culture and will grow to high density in Expi293™ Expression Medium. Expi293F™ Cells are highly transfectable and generate superior protein yields compared to standard 293 cell lines in transient protein expression.

Expi293F™ GnTI- Cells

Expi293F™ GnTI- Cells are derived from Expi293F™ Cells and have been engineered to lack N-acetylglucosaminyl-transferase I (GnTI) enzyme activity leading to the production of glycoproteins with a uniform $(GlcNAc)_2Man5$ glycopattern.

Expi293F™ Inducible Cells

Expi293F™ Inducible Cells are derived from Expi293F™ Cells and stably express high levels of the tetracycline repressor protein (TetR) from the pcDNA™6/TR plasmid. When used with compatible inducible vectors (e.g. pcDNA™5/TO Mammalian Expression Vector), the gene of interest is repressed with very low levels of basal expression until induction with tetracycline. Expression can be modulated by addition of different amounts of tetracycline. For best results, Expi293F™ Inducible Cells should be maintained under selective pressure of blasticidin. For further details, see "Guidelines for inducible cells" on page 2.

Expi293F™ Inducible GnTI- Cells

Expi293F™ Inducible GnTI- Cells are derived from Expi293F™ GnTI- Cells and stably express high levels of the tetracycline repressor protein (TetR) from the pcDNA™6/TR plasmid in combination with knockout of the GnTI gene. When used with compatible inducible vectors (e.g. pcDNA™5/TO Mammalian Expression Vector), the gene of interest is repressed with very low levels of basal expression until induction with tetracycline. Expression can be modulated by addition of different amounts of tetracycline. Resultant glycan patterns of the expressed glycoprotein are consistent with those expressed by Expi293F™ GnTI- Cells. For best results, Expi293F™ Inducible GnTI- Cells should be maintained under selective pressure of blasticidin. For further details, see "Guidelines for inducible cells" on page 2.

Contents and storage

Table 1 Expi293F™ cell lines

Contents	Amount	Cat. No.	Storage
Expi293F™ Cells[1] (1 × 10^7 cells/mL)	1 mL	A14527	Vapor phase, liquid nitrogen[2]
	6 × 1 mL	A14528	
Expi293F™ GnTI- Cells[1] (1 × 10^7 cells/mL)	1 mL	A39240	
Expi293F™ Inducible Cells[1] (1 × 10^7 cells/mL)		A39241	
Expi293F™ Inducible GnTI- Cells[1] (1 × 10^7 cells/mL)		A39242	

[1] Cells are cryopreserved in 90% Expi293™ Expression Medium and 10% DMSO.
[2] Store the frozen cells in vapor phase liquid nitrogen immediately upon receipt and until ready to use. Do not store the cells at –80°C.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. Or other major laboratory suppliers (MLS).

Table 2 For all Expi293F™ cell lines

Item	Source
Expi293™ Expression Medium	A1435101
Nalgene™ Single-Use PETG Erlenmeyer Flasks with Plain Bottom: Sterile	4115-0125
MaxQ™ HP Tabletop Orbital Shaker	SHKE416HP
CO_2 controlled incubator	MLS
Reagents and equipment to determine cell number and viability: • Hemocytometer with trypan blue • Cell counter	 • MLS • MLS

Table 3 For Expi293F™ Inducible Cells and Expi293F™ Inducible GnTI- Cells

Item	Source
pcDNA™5/TO Mammalian Expression Vector	V103320
Blasticidin S HCl (10 mg/mL)	A1113903
Tetracycline Hydrochloride	A39246

Culture conditions

Media: Expi293™ Expression Medium

Cell line: Expi293F™ cell lines

Culture type: Suspension

Shake flask type: It is recommended to use PETG or polycarbonate, non-baffled, vented Erlenmeyer flasks; however, baffled Erlenmeyer flasks can also be used.

Temperature range: 37°C ±0.5°C

Shaker speed: For shakers with a 19-mm shaking diameter, set the shake speed to 125 ±5 rpm. For shakers with a 25-mm shaking diameter, set the shake speed to 120 ±5 rpm. For shakers with a 50-mm shaking diameter, set the shake speed to 95 ±5 rpm.

Incubator type: ≥80% humidified, 8% CO_2 atmosphere. Ensure proper gas exchange and minimize exposure of culture to light.

General cell handling

- All solutions and equipment that come in contact with the cells must be sterile. Always use proper aseptic technique and work in a laminar flow hood.
- For all cell manipulations, mix the cells by gentle swirling and avoid vigorous shaking and pipetting. Cell health is critical for maximal performance.
- Expi293F™ cell lines are robust cell lines adapted to high-density growth conditions with a doubling time of approximately 24 hours during log phase growth.

Guidelines for thawing and storing cells

- On receipt, either thaw the cells immediately into pre-warmed Expi293 Expression Medium or immediately place the frozen cells into vapor phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term, extreme temperature changes. When storing cells in liquid nitrogen following receipt on dry ice, allow the cells to remain in liquid nitrogen for 3–4 days before thaw.
- Before starting experiments, ensure to have cells that are established and have frozen stocks on hand. On receipt, grow and freeze multiple vials of Expi293F™ cells to ensure that you have an adequate supply of early-passage cells.

Guidelines for cell maintenance and subculturing

- Allow freshly thawed cells to recover in culture for three or more passages post-thaw before transfecting.
- Use an automated cell counter or a hemocytometer with the trypan blue exclusion method to determine cell viability. Log phase cultures should be ≥95% viable.
- When thawing or subculturing cells, transfer cells into pre-warmed medium.
- Cell viability should be ≥90% within 4–7 days post-thaw with viable cell density typically >1 × 10^6 viable cells/mL at this time; if viability is not ≥90%, cells should be incubated for up to an additional 3 days in order to reach this criterion.
- At the time of first subculture, cells should be subcultured when the viable cell density reaches 1–3 × 10^6 viable cells/mL.
- For general maintenance of cells, passage Expi293F™ cells when they reach a density of approximately 3–5 × 10^6 viable cells/mL (i.e., early log-phase growth), typically every 3–4 days.

  Note: Cells that are subcultured at densities outside of this early log-phase growth window can show longer doubling times and lower protein titers over time. Modify the initial seeding density to attain the target cell density of 3–5 × 10^6 viable cells/mL at the time of subculturing.

Guidelines for inducible cells

For Expi293F™ Inducible Cells or Expi293F™ Inducible GnTI- Cells:

- For routine culture maintenance, add blasticidin to a final concentration of 20 µg/mL to culture medium.
- Blasticidin can be present in the media during cryopreservation without impacting cell health.
- An inducible expression vector must be utilized in conjunction with the Expi293F™ Inducible Cells and Expi293F™ Inducible GnTI- Cells. pcDNA™5/TO expression vector is recommended for lowest levels of basal expression and highest levels of expression upon induction with tetracycline.
- We recommend making a 1 mg/mL tetracycline stock solution in water.

Thaw Expi293F™ cells

1. Remove one vial of cells from liquid nitrogen, then swirl in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains.

   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 laminar flow hood.

3. Use a 2-mL or 5-mL pipette, to transfer the entire contents of the cryovial into a 125-mL polycarbonate or PETG, disposable, sterile, vented Erlenmeyer shaker flask containing 30 mL of pre-warmed Expi293™ Expression Medium.

4. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform according to the following table.

Shaker diameter	Shake speed (rpm)
19 mm	125 ± 5
25 mm	120 ± 5
50 mm	95 ± 5

5. Allow cells to culture for 3–4 days post-thaw, then determine viable cell density and percent viability.

   Note: At 24 hours post-thaw, viability may drop to 80%, but should not get below 70%. It can take up to 7 days for cells to recover and reach ≥90% viability post-thaw.

6. Perform the first subculture when the viable cell density reaches 1–3 × 10^6 viable cells/mL (typically 4–7 days post-thaw).

Subculture Expi293F™ cells

1. Use the viable cell density to calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities in Table 4 and the recommended culture volumes in Table 5.

   Table 4 Recommended seeding densities for routine cell culture maintenance

Sub-culture timing	Recommended seeding density
For cells ready 3 days post-subculture	0.4–0.5 × 10^6 viable cells/mL
For cells ready 4 days post-subculture	0.3–0.4 × 10^6 viable cells/mL

   Table 5 Recommended volumes for routine cell culture maintenance in vented, non-baffled flask

Flask size	Culture volume
125 mL	30–35 mL
250 mL	60–70 mL
500 mL	100–120 mL
1 L	220–240 mL
2 L	440–480 mL
3 L	800–1,000 mL

2. Transfer the calculated volume of cells to fresh, pre-warmed Expi293™ Expression Medium in a shake flask.

3. Incubate flasks in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform until cultures reach a density of 3–5 × 10^6 viable cells/mL.

   Note: Do not let cells grow above 5 × 10^6 viable cells/mL during routine culture.

   Note: Cells that are subcultured at densities outside of this early log-phase growth window can show longer doubling times and lower protein titers over time. Modify the initial seeding density to attain the target cell density of 3–5 × 10^6 viable cells/mL at the time of subculturing.

4. Repeat step 1 to step 3 to maintain or expand the cells for experiments.

   Note: It is recommended to discard cells and start a new culture after passage number 30.

Cryopreserve Expi293F™ cells

1. Centrifuge the cells at 300 × *g* for 5 minutes to collect the cells at the bottom.

2. Discard the supernatant, then gently resuspend the cells in ice cold Expi293™ Expression Medium containing 10% DMSO by pipetting up and down.

3. Dilute the cells in fresh Expi293™ Expression Medium containing 10% DMSO to a final density of 1 × 10^7 viable cells/mL.

   Note: Alternatively, conditioned medium obtained following centrifugation of the cells before freeze down can be added to fresh Expi293™ Expression Medium in the following ratios: 45% fresh Expi293™ Expression Medium, 45% conditioned medium, and 10% DMSO to generate a conditioned freeze medium.

4. Freeze the cells in an automated or manual controlled-rate freezing apparatus following standard procedures.

   For ideal cryopreservation, the freezing rate is a decrease of 1°C per minute.

5. Transfer frozen vials to vapor phase liquid nitrogen for long-term storage.

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