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 optimized to grow to high density in Expi293™ Expression Medium. Expi293F™ Cells are highly transfectable and generate superior transient 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. Add 30 mL of pre-warmed Expi293™ Expression Medium to a 125-mL Erlenmeyer shaker flask.

2. Remove a vial of Expi293F™ cells from liquid nitrogen and swirl gently in a 37°C water bath for 1 to 2 minutes to thaw the cells rapidly until only a small amount of ice remains.

   Note: Do not submerge the vial in the water.

3. Just before the cells are completely thawed, decontaminate the vial with 70% ethanol before opening it in a laminar flow hood.

4. Gently invert the cell vial to mix the contents. Uncap the cell vial and transfer 50 µL of cells into 450 µL of Ca^{2+}/Mg^{2+} free PBS for viability and viable cell density determination by trypan blue dye exclusion assay.

   Note: Trypan blue may interact with components in cell culture media leading to aggregation that can be misinterpreted as dead cells using typical cell counting instruments and algorithms. Dilution with PBS is not required during routine cell culture maintenance.

5. Using a 1-, 2-, or 5-mL pipette, gently transfer the remaining cell volume drop wise to the shake flask containing the pre-warmed culture medium prepared in step 1.

6. Incubate the cells in a 37°C incubator with ≥80% relative humidity and 8% CO_2 on an orbital shaker platform. The use of non-humidified incubators is not recommended due to the significant loss of volume in the culture flasks by evaporation.

   Note: Set the shake speed to 125 ±5 rpm for shakers with a 19-mm shaking diameter, 120 ±5 rpm for shakers with a 25-mm shaking diameter and 95 ±5 rpm for shakers with a 50-mm shaking diameter.

7. Three to four days post-thaw, determine the viable cell density and percent viability. Cell viability should be ≥90% with a viable cell density >1 × 10^6 viable cells/mL.

   Note: If the viability is <90% on days 3 to 4 post-thaw, cells may be cultured for up to an additional 3 days in order to reach the desired viability. Cells should not be subcultured until viable cell density reaches >1 × 10^6 viable cells/mL.

8. For subsequent routine cell culture maintenance, subculture cells every 3 to 4 days when the viable cell density reaches 3 × 10^6 to 5 × 10^6 viable cells/mL.

   Note: Do not subculture cells before reaching early log phase growth of 3 × 10^6 viable cells/mL. Similarly, do not let cells overgrow above 5 × 10^6 cells/mL. Modify the initial seeding density to attain target cell density of 3 × 10^6 to 5 × 10^6 viable cells/mL at the time of subculturing.

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

Expi293F™ cells can be frozen directly in Expi293™ Expression Medium with 7.5% DMSO. Alternatively, conditioned cryopreservation medium consisting of 42.5% fresh Expi293™ Expression Medium, 50.0% conditioned medium and 7.5% DMSO can be used.

Note: It is critical that chemical compatibility be maintained throughout the freezing process to eliminate the potential for plastics leachables/extractables to negatively impact cell health. For all steps where DMSO is present (with exception only to the pipet tips used for aliquoting the final cell solution into cryo-vials), glass serological pipettes are suggested to be used, as polystyrene is generally not compatible with concentrated DMSO. Similarly, DMSO containing freeze medium must be prepared in polypropylene or other known DMSO compatible bottles and the final cell suspension in freeze medium must be prepared in polypropylene or other known DMSO compatible bottles prior to aliquoting into polypropylene freeze tubes. Additionally, all pipets and bottles may be rinsed with sterile water, PBS or culture medium before use as desired. Refer to **https://tools.thermofisher.com/content/sfs/brochures/D20480.pdf** for guidance on chemical compatibility.

1. Determine the density and volume of cells required for banking cells at a final density of 1×10^7 viable cells/mL in 1.1 mL total volume. Expand the cells, maintaining a viable cell density of $3 \times 10^6 – 5 \times 10^6$ viable cells/mL, until the desired volume of cells to be banked is ready. Do not use shake flasks larger than 2 L to culture the cells, as these flasks differ in shape and require altered shaking speeds and be sure to adhere to the shaking speed, orbital diameter and maximum flask volume recommendations.

2. While expanding the cells for banking, prepare an additional flask which will be used to generate conditioned medium. This flask should be prepared and expanded in the same manner as the flasks used for cell banking.

   Note: Based on the total volume of the bank, prepare at least ½ volume conditioned medium (i.e., If total volume for cell banking is 100 mL, at least 50 mL of conditioned medium will be required).

3. Prepare labels and label the appropriate number of vials. If vials are labeled on a day other than that of the harvest, store vials in a biosafety cabinet.

   Note: To reduce the risk of damaging the cells during freezing procedures, cells pellets will be resuspended in cold, 100% conditioned medium followed by 1:1 addition of cold freeze medium with 15% DMSO to reach a final concentration of 7.5% DMSO, 50% conditioned medium, and 42.5% fresh medium. It is critical to resuspend the cell pellets in cold medium free of DMSO.

   Note: If the use of 100% fresh culture medium for banking cells is desired, cells pellets will be resuspended in cold, 100% fresh medium followed by a 1:1 addition of cold, 15% DMSO in fresh cell culture medium to reach a final concentration of 7.5% DMSO and 92.5% fresh medium.

Prepare conditioned medium

All conditioned medium is to be pre-chilled before use in cell banking.

1. Remove conditioned medium flask from incubator and transfer the entire volume of cell suspension into a sterile polypropylene centrifuge tube or bottle.

2. Centrifuge the cells at 300 × *g* for 10 minutes at 2–8°C.

3. Carefully decant the supernatant into a sterile polypropylene bottle without disturbing the cell pellet; the decanted supernatant will be used as the conditioned medium.

4. Store the conditioned medium in a 2–8°C refrigerator or on ice for a minimum of 2 hours.

5. Discard the cell pellets.

Prepare freeze medium (2X)

1. In a sterile polypropylene bottle, prepare the required amount of fresh culture medium supplemented with 15% DMSO. This represents a 2X freeze medium. It is recommended to use glass serological pipettes for transferring the concentrated DMSO to the culture medium. Keep 2X DMSO freeze medium cold at 2–8°C or on ice until use.

2. Remove calculated volume of cells from incubation and transfer into sterile polypropylene centrifuge bottle/tube. Centrifuge the cells at 200 × *g* for 10 minutes at 2–8°C. Carefully decant the supernatant without disturbing the cell pellets. After removing the supernatant, gently flick the bottom of the centrifuge tube to loosen the cell pellet.

3. Resuspend the cell pellet by gently pipetting with ~10% volume of conditioned medium using a wide bore pipet (i.e., If total bank volume is 200 mL, use 20 mL to resuspend cell pellet).

4. Add additional conditioned medium to the centrifuge bottle to obtain a 2X cell stock. For example, if banking at 1×10^7 cells/mL prepare a 2X cell stock at 2×10^7 cells/mL. Gently swirl the bottle to ensure a homogenous mixture and keep cold on ice or cold blocks.

   Note: It is critical that the next steps are performed as quickly as possible to limit the DMSO exposure time.

5. Using a glass serological pipette, add the calculated volume of cold, 2X freeze medium to the bottle containing 2X cell stock in conditioned medium from step 4.

6. Gently swirl the bottle to ensure a homogenous mixture and keep cold on ice or cold blocks.

   At this point the volume should be equal to the total bank volume at a final concentration of 7.5% DMSO, 50% conditioned medium, and 42.5% fresh medium with a cell density of 1×10^7 cells/mL.

7. Immediately dispense a 1.1 mL aliquot of the final cell suspension from step 6 into labeled cryo-vials using a repeater pipette or sterile serological pipettes.

8. Gently swirl the cell suspension to mix before each refill of the multi-dispenser pipette or serological pipette. Make sure to keep the cell suspension cold during the entire aliquoting process.

   Note: The DMSO in the 2X freeze medium is harmful to the cells so it is important to limit the amount of DMSO exposure prior to freezing. We recommend keeping the DMSO exposure time ≤ 60 minutes as possible and keeping all reagents cold during this time.

9. Transfer the cryo-vials to isopropanol containing cryo-freezing chambers and store at -80°C for 24–48 hours. Do not store cells at -80°C for more than 48 hours. After 24–48 hours transfer cells to final storage in vapor phase liquid nitrogen.

   This freezing regimen approximates a 1°C per minute cooling in the isopropanol containers to -80°C followed by a 2°C per minute cooling in vapor phase liquid nitrogen to final storage temperature. These cooling conditions may be utilized as a basis for controlled rate freezing protocol design.

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Revision	Date	Description
C.0	10 February 2023	Updated thaw and cryopreserve protocols.
B.0	14 April 2019	Revision.
A.0	31 March 2015	New document for Expi293F™ cell lines.

The information in this guide is subject to change without notice.

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