

Express Five™ SFM

Description

Express Five™ Serum Free Medium (SFM) is a serum-free, protein-free insect cell medium developed for maximum cell growth and recombinant protein expression using the Baculovirus Expression Vector System (BEVS) in the *Trichoplusia ni* BTI-TN-5B1-4 (High Five™) cell line. Express Five™ SFM allows long-term cell growth and outperforms other commercially available serum-free and serum-supplemented media achieving High Five™ cell densities of up to 6×10^6 cells/mL and recombinant protein expression levels up to 10-fold higher. Express Five™ SFM is formulated without L-glutamine to maximize shelf life.

Product	Catalog No.	Amount	Storage	Shelf life*
Express Five™ SFM (1X), liquid	10486-025	1000 mL	2°C to 8°C; Protect from light	18 months

*Shelf life duration is determined from Date of Manufacture.

Important information

The color of Express Five™ SFM may vary between manufactured lots due to the variability in carbohydrate processing of a critical biologically active growth promoting component. The difference in coloration has no impact on medium performance.

Safety information

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

Prepare media

Express Five™ SFM requires supplementation with 18 mM L-glutamine. Addition of a surfactant such as Pluronic™ F-68 is not required.

1. Aseptically add 90 mL of 200 mM L-glutamine per 1000 mL of Express Five™ SFM before use.
2. If L-glutamine is not required, add 90 mL of sterile distilled water, and adjust the osmolality to 365 mOsm using a sterile solution of 15% NaCl.

Note: There is ample room in the bottle for this supplementation.

Note: Store complete Express Five™ SFM in the dark at 2°C to 8°C.

Culture conditions

Media: Express Five™ SFM

Cell line: BTI-TN-5B1-4 *Trichoplusia ni* (High Five™) cell line

Culture type: Suspension or Adherent

Culture vessel: shake flask, spinner bottle or T-flask.

Temperature range: 27°C to 28°C

Incubator atmosphere: non-humidified, air regulated non-CO₂ atmosphere. 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. Swirl contents of vial while thawing and remove vial from the water bath when the last trace of ice has melted.
2. Transfer entire contents of the cryovial into the appropriately sized vessel so that the cells are seeded at 5×10^5 cells/mL of pre-warmed complete Express Five™ SFM.
3. Incubate at 27–28°C in a non-humidified, air regulated non-CO₂ atmosphere, on an orbital shaker platform rotating at 120–140 rpm. Loosen flask caps to allow for gas exchange.
4. Dilute cell suspension to 5×10^5 viable cells/mL when viable cell density reaches $\geq 1 \times 10^6$ cells/mL for the first two passages, thereafter subculture when cells reach $>2 \times 10^6$ viable cells/mL.

Note: It is recommended to thaw a fresh low-passage vial of cells every 3 months or 30 passages.

Subculture suspension cultures

Insect cells are sensitive to physical shearing. If using spinner flasks, ensure that impeller mechanisms rotate freely and do not contact vessel walls or base (adjust prior to autoclaving).

1. If cell clumping is observed disperse clumps into a single cell suspension by following step 4 *Subculture Monolayer Cultures*.
2. Determine viable cell density using a Countess™ Automated Cell Counter or alternative automated or manual method.
3. Seed cells at 3×10^5 – 5×10^5 viable cells/mL in sterile culture vessels containing pre-warmed Express Five™ SFM. (30 mL per 125-mL shake flask, 75–100 mL per 100-mL spinner bottle).
4. Incubate at 27°C to 28°C in a non-humidified, air regulated non-CO₂ atmosphere. Loosen caps to allow for gas exchange.
5. Rotate shake flask cultures on an orbital shaker platform at 120–140 rpm, set impeller stirring rate to 75 rpm (optimum impeller speed must be empirically determined for each spinner apparatus) for spinner bottles.
6. Subculture cells when viable cell density reaches $>2 \times 10^6$ viable cells/mL (about twice a week) into clean, sterile flask(s) with fresh pre-warmed Express Five™ SFM.

Note: To reduce accumulation of cell debris and metabolic waste by-products in suspension cultures, gently centrifuge the cell suspension at $100 \times g$ for 5 minutes and gently resuspend pellet in fresh Express Five™ SFM once every 2–3 weeks.

Subculture monolayer cultures

1. Observe cell monolayer to ensure 80–90% confluence. Aspirate, and discard, spent medium and floating cells from a confluent monolayer.
2. Add 4 mL (per 25 cm²) pre-warmed Express Five™ SFM to the flask and resuspend cells by repeatedly streaming the medium across the monolayer.
3. Observe cell monolayer to ensure cell detachment from the surface of the flask. Firmly rap the side of the flask on the palm of your hand or a hard flat surface if necessary.
4. Transfer entire cell suspension from the flask to a sterile conical tube; any cell clumps quickly settle to the bottom after 1–2 minutes. Pipet the clumps into a 10-mL pipette and gently break up the clumps by pressing the pipette tip against the bottom of the tube and gently expel the cells back into the medium, repeat 1–2 more times if necessary to break up remaining clumps. Too harsh pipetting will decrease cell viability due to sensitivity of cells to shear force.
5. Determine viable cell density using a Countess™ Automated Cell Counter or alternative automated or manual method.
6. Seed culture flasks at 2×10^4 – 5×10^4 viable cells/cm².

- Incubate at 27°C to 28°C in a non-humidified, air regulated non-CO₂ atmosphere. Loosen caps to allow for gas exchange.
- Exchange spent media with fresh pre-warmed complete Express Five™ SFM every 2–3 days and passage when cell monolayer reaches ~90% confluence.

Note: BTI-TN-5B1-4 (High Five™) cells are not anchorage dependent and may be transferred between monolayer and spinner/shaker culture repeatedly without noticeable perturbation of normal viability, morphology, or growth rate when seeded at the recommended densities.

Adapt BTI-TN-5B1-4 (High Five™) cells to Express Five™ SFM

We recommend using sequential adaptation of monolayer cultures when adapting High Five™ cells to Express Five™ SFM. It is critical that cell viability be at least 90% and the growth rate be in mid-logarithmic phase prior to initiating adaptation procedures.

- Begin with a culture at 50% confluency. Exchange serum-supplemented media for fresh pre-warmed 25:75 ratio of Express Five™ SFM to the original media.
- Incubate at 27°C to 28°C in a non-humidified, non-CO₂ atmosphere until cells reach 90–100% confluency. Loosen caps to allow for gas exchange.
- Subculture cells following steps 1–4 *Subculture Monolayer Cultures* using a 50:50 ratio of Express Five™ SFM to original medium. Seed culture vessels at 50% confluency (e.g., return one half volume of the cell suspension and add an equal volume of 50:50 ratio media).
- Repeat step 2–3, increasing stepwise the ratio of Express Five™ SFM to original medium (75:25 followed by 90:10) until the cells are subcultured into 100% Express Five™ SFM.
- After several passages in 100% Express Five™ SFM with consistent growth the culture is considered to be adapted to Express Five™ SFM.

Cryopreservation

- Prepare the desired quantity of cells in either a spinner or shaker culture, harvesting in mid-log phase of growth with viability >90%.
- Determine the viable cell density and calculate the required volume of cryopreservation medium required to yield a final cell density of 0.5×10^7 – 1×10^7 cells/vial.
- Prepare the required volume of cryopreservation medium of 92.5% Express Five™ SFM (50:50 ratio of fresh to conditioned media) + 7.5% DMSO on day of intended use, store at 4°C until use.
- 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.
- Dispense aliquots of this cell suspension into cryovials according to the manufacturer's specifications.
- Cryopreserve in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
- Transfer frozen cells to liquid nitrogen, (vapor phase) storage at –200°C to –125°C is recommended.

Related products

Product	Catalog No.
High Five™ Cells, SFM Adapted	B855-02
L-Glutamine 200mM (100X), liquid	25030
Penicillin-Streptomycin, liquid	15070
Bac-to-Bac™ Baculovirus Expression System	10359
Bac-to-Bac™ Vector Kit	10360
BaculoDirect™ N-Term Expression Kit	12562-054
BaculoDirect™ N-Term Transfection Kit	12562-062
BaculoDirect™ C-Term Expression Kit	12562-013
BaculoDirect™ C-Term Transfection Kit	12562-039
Bac-N-Blue™ Transfection Kit	K855-01
Trypan Blue Stain	15250
Countess™ Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

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

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.

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