Guidelines for the use of insect cell culture medium

Version 2.0

We have two types of serum-free, animal-component-free basal medium for insect cell culture: IT SFM 03 and Insect SFM 1407. They support high-density suspension culture of insect cells such as *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (H5), as well as high-efficiency protein expression in the baculovirus expression system. For H5 cells expressing PCV2, in general, Insect SFM 1407 medium is recommended for cell growth and cultivation, while IT SFM 03 medium is the preferred choice for viral protein expression.

Product	Application	Catalog no.	Form	Package sizes
Insect SFM 1407	Serum-free medium for insect cell culture	11003-1407	Dry powder	2 L, 10 L, 100 L, Customized
		11001-14070	Liquid	500 mL, 1000 mL
TE 031	Used in conjunction with Insect SFM 1407	99174-23065	Liquid	6 mL, 15 mL, 150 mL
IT SFM 03	Serum-free medium for insect cell culture	11009-1353.2	Dry powder	2 L, 10 L, 100 L, Customized
		11009-23027.2	Liquid	500 mL, 1000 mL
IT F 01	Used in conjunction with IT SFM 03	99150-22063.1	Liquid	6 mL, 15 mL, 150 mL

Component Information

All products don't contain any components of animal origin, proteins, undefined lysates, phenol red, and EDTA. The medium contains L-glutamine and glucose.

Safety Warning

Read the Material Safety Data Sheets (MSDS) and follow handling instructions. Wear appropriate personal protective equipment, including eyewear, clothing, and gloves.

Intended Use

For research and further manufacturing use only.

Storage and Stability

Liquid Medium

The liquid medium should be stored at 2–8℃ and protected from light. The product is stable for 12 months when unopened and stored properly. Addition of other supplements may affect storage conditions and shelf life. Do not use the medium if particulate matter or cloudiness is observed.

Powder Medium

The dry powder medium should be stored at 2–8℃ and protected from light and moisture. The product is stable for 24 months when unopened and stored properly. This product is highly hygroscopic, ensure the container is kept tightly sealed in a dry environment.

Prepare Liquid Medium from Dry Powder

Insect SFM 1407 (11003-1407)

1. Fill the mixing container with purified water (room temperature) about 80% of the final volume, start stirring.
2. Slowly add 45.52 g/L of dry powder medium. Mix until there is no dry powder on the surface of the solution. Mix for 30 minutes.
3. Adjust the pH to 6.0–6.2 with 1 M–5 M HCl or NaOH solution. Mix for 10 minutes. The pH may rise 0.1 to 0.2 after sterile filtration.
4. Add 1.0 mL/L of the sterile-filtered TE031 (99174-23065). Mix for 10 minutes.
5. Adjust the final volume with purified water. Mix for 10 minutes.
6. Filter the medium using a membrane filter with 0.22 μm pore size immediately.

Note：When adding TE031 in Step 4, pour the TE031 solution in one portion directly into the stirring vortex.

IT SFM 03 (11009-1353.2)

1. Fill the mixing container with purified water (room temperature) about 90% of the final volume with purified water (room temperature), start stirring.
2. Slowly add 44.13 g/L of dry powder medium. Mix until there is no dry powder on the surface of the solution and stir for 10 minutes.
3. Adjust the pH to 6.0–6.2 with 1 M–5 M HCl or NaOH solution. Mix for 20 minutes.
4. Add 0.5 mL/L IT F 01 (99150-22063.1). Mix for 10 minutes.
5. Adjust the final volume with purified water (Solution density:1.01 g/mL). Mix for 10 minutes.
6. Filter the medium immediately using a membrane filter with 0.22 μm pore size.

Culture Conditions

Culture Type: Suspension
Culture Vessels: TPP tubes and shake flasks
Shaking Speed: For TPP tubes, it is recommended 200 rpm @50 mm orbital diameter; For shake flasks, it is recommended 125 rpm @50 mm orbital diameter or 130 rpm@25mm orbital diameters.
Culture Temperature: 27℃
CO_2 Concentration: 0%
Relative Humidity: 80% RH

Recover Cells

- **Thawing:** Rapidly thaw the cryopreserved vial in a 37℃-water bath for 1 to 2 minutes.
- **Centrifugation and Resuspension:**
  a. Aseptically transfer the entire cell contents into a sterile centrifuge tube containing 30 mL of pre-warmed Insect SFM 1407 medium.
  b. Centrifuge the cells at 1000 rpm (about 233 × g) for 5 minutes.
  c. carefully discard the supernatant, resuspend the cell pellet in a required volume of pre-warmed Insect SFM 1407 medium to achieve a final seeding density of (0.8–1.2) × 10^6 cells/mL.
  d. Transfer the cell suspension into a new TPP tube or shaker flask.
- **Initial Culture:** Incubate the cells under the conditions specified in the "**Culture Conditions**" section.
- **Routine Passaging:** Beginning with the first full passage, subculture the Sf9 cells every 72 ± 2 hours, and the H5 cells every 48 ± 2 hours by following the steps outlined in "**Passage Cells and Scale-up**".

Passage Cells and Scale-up

- **Pre-Passage Assessment:** Cell density and cell viability are determined for Sf9 cells at 72 ± 2 hours and H5 cells at 48 ± 2 hours after the previous subculture.
- **Passaging Criteria and Execution:**
  a. Sf9 cells：When the viable cell density reaches at least 6.0 × 10^6 cells/mL with viability exceeding 90%, subculture the cells by seeding them at a density of (0.8–1.2) × 10^6 cells/mL in fresh Insect SFM 1407 medium. If the viable cell density is less than 6.0 × 10^6 cells/mL or cell viability drops below 90%, perform centrifugal subculture, centrifuge the cell suspension at 1,000 rpm (approximately 233 × g) for 5 minutes, discard the supernatant, and subculture at a seeding density of (0.8–1.2) × 10^6 cells/mL.
  b. H5 cells：When the viable cell density reaches at least 4.0 × 10^6 cells/mL with viability exceeding 90%, subculture the cells by seeding them at a density of (0.8–1.2) × 10^6 cells/mL in fresh Insect SFM 1407 medium. If the viable cell density is less than 4.0 × 10^6 cells/mL or cell viability drops below 90%, perform centrifugal subculture, centrifuge the cell suspension at 1,000 rpm (approximately 233 × g) for 5 minutes, discard the supernatant, and subculture at a seeding density of (0.8–1.2) × 10^6 cells/mL.
  c. Culture the newly seeded cells according to the **"Culture Conditions".**
- **Adaptation Requirement:** Prior to use in other applications, cells must undergo a minimum of 3 consecutive, successful passages in Insect SFM 1407 medium to ensure proper adaptation.

Adaptation to Insect SFM 1407 Medium

Direct Adaptation

- **Initial Seeding:** Directly transfer cells from their current medium into Insect SFM 1407 medium at a seeding density of (0.8–1.2) × 10^6 cells/mL. Culture the cells according to the specifications in the "**Culture Conditions**" section.
- **Monitoring:** Following the initial transfer, it is recommended to monitor cell density (× 10^6 cells/mL) and viability (%) daily.
- **Success Criterion:** Adaptation is considered successful when, after several consecutive passages, the cell growth profile (including doubling time and maximum density) aligns consistently or beyond with the historical data from the client's existing process.
- **Passaging and Other Experiments:**
  a. Perform routine passaging by following the procedures outlined in "Passage Cells and Scale-up".
  b. Cells should undergo a minimum of 3 to 5 consecutive, stable passages in Insect SFM 1407

medium before being used for other experiments.

Sequential Adaptation

- **Initial Seeding:** Seed insect cells at an initial viable cell density of (0.8–1.2) × 10^6 cells/mL using a blend of basal original medium and Insect SFM 1407.
- **Gradual Medium Transition:** At each successive passage, gradually lower the percentage of original medium and raise the proportion of Insect SFM 1407 following the gradient ratios below:
  90:10 (Passage 1) → 75:25 (Passage 2) → 50:50 (Passage 3) → 25:75 (Passage 4) → 0:100 (Passage 5, full replacement with Insect SFM 1407).
- **Process Alignment & Verification:**
  a. When cell growth curve matches the growth trend specified in the customer's standard process, perform another 2–3 consecutive passages under identical culture conditions.
  b. Advance to subsequent steps only if steady, reproducible cell growth is achieved during verification culture.
- **Final Adaptation in 100% Complete Medium:** Maintain serial subculture in 100% Insect SFM 1407 medium. Adaptation is deemed complete once the cell growth performance consistently meets or exceeds the customer's benchmark, normally after a minimum of 3–5 stable passages in full Insect SFM 1407. Cells can then be applied for downstream experiments.

Cryopreservation

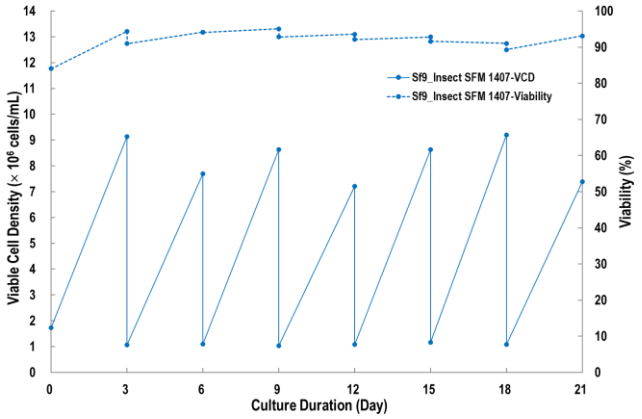
- **Recommended Cell State：**
  Use cells at mid-logarithmic growth phase (approximately 48 ± 2 hours post seeding) with viability above 95%.
- **Procedure：**
  Prepare cryopreservation medium consisting of 60% Insect SFM 1407, 30% serum and 10% DMSO; store at 2–8℃ prior to use.
  a. Determine cell density and cell viability, then calculate the required medium volume to adjust cells to a final concentration of 3.0 × 10^7 cells/mL.
  b. Harvest cells via centrifugation (1,000 rpm ≈ 233 × g, 5 min), discard supernatant, and resuspend cell pellet in pre-chilled cryopreservation medium at the calculated volume.
  c. Dispense 1 mL cell suspension into each cryovial, load vials into pre-cooled controlled-rate freezing containers, then store at −80℃ for no less than 24 h.
  d. For long-term preservation, transfer cryovials to the vapor-phase of liquid nitrogen storage tank.

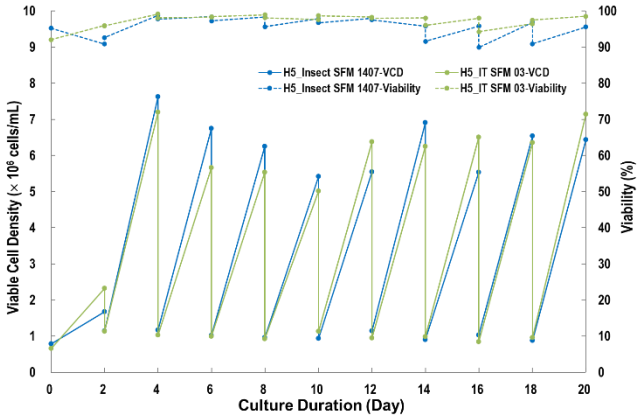
Protein Expression (PCV2 case study)

- Express timing: Sf9 cells on Day 3 post cell culture; H5 cells on Day 2 post cell culture.
- Infection density: cells are cultured in Insect SFM 1407 medium, dilute the culture with fresh IT SFM 03 medium to the target infection density of (1.0–2.5) × 10^6 cells/mL. The optimal infectious cell density should be determined experimentally for each individual virus.
- Recommended Virus inoculation dose: (0.01–0.1) MOI. The optimal dose should be determined experimentally for each individual virus.
- Viral infection: Cells in logarithmic growth phase are diluted to infectious density with fresh medium. Calculate required virus volume based on infection ratio, add virus into cell suspension, then incubate for 5–10 days under specified culture conditions. Set multiple sampling time points during cultivation to monitor viral expression and confirm the optimal harvest time.

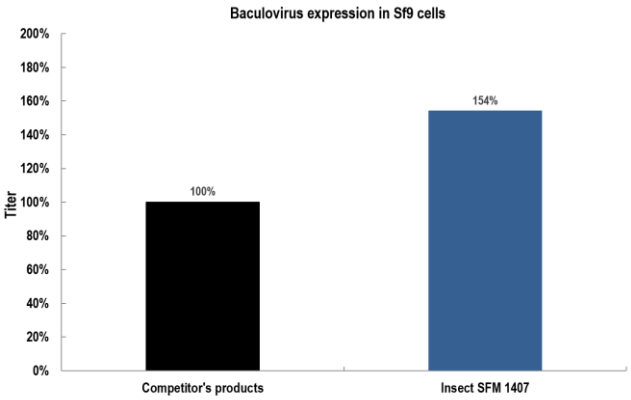
Appendix

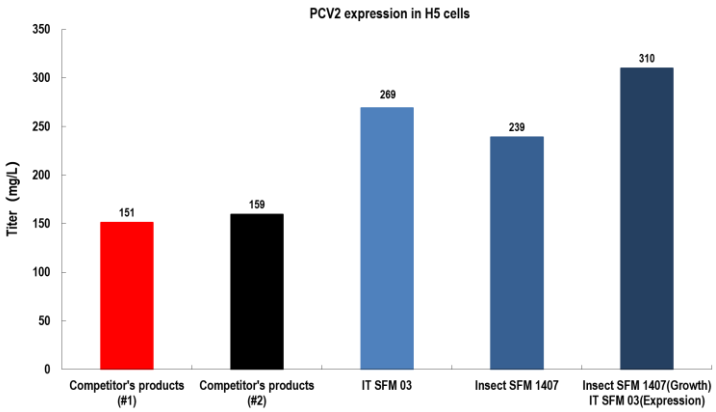
- **Growth data of Sf9 and H5 cells**





- **Expression data of Sf9 and H5 cells**





Related Products

Product	Catalog No.
Glucose (300 g/L)	99024-16023
L-Glutamine (200 mM)	99025-17029
0.25% Trypsin-EDTA	99008-14020