FUJIFILM IrvineScientific

IS Sf Insect

Catalog #	Product	Size
91174	IS Sf Insect	1 L liquid

Intended Use

For research or further manufacturing use only.

Product Description

IS Sf Insect is a serum-free, animal component-free medium designed to support suspension culture of Sf9 and Sf21 insect cells for the production of recombinant proteins, including viral-like particles, and viral vectors using Baculovirus Expression Vector Systems (BEVS). Developed for *Spodoptera frugiperda* (Sf9, Sf21) cells, IS Sf Insect contains L-Glutamine and Kolliphor® P188, and is a ready-to-use medium for long-term suspension culture.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis (COA) which is available upon request.

Storage Instructions and Stability

Handle using aseptic techniques to avoid contamination. Store at 2-8°C, protected from light. This product is stable for 12 months, when unopened and properly stored. Do not use after the assigned expiration date. Not validated for use beyond the unopened expiry shelf life. Do not use any bottle of medium that shows evidence of particulate matter or cloudiness.

Precautions

This product is for research use or further manufacturing use only. Not for injection or diagnostic procedures. The safety and efficacy of this product in diagnostic or other clinical uses has not been established. This reagent should not be used beyond twelve months indicated in the storage instructions. Please refer to the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Directions for Use

MEDIUM SUPPLEMENT

This medium comes supplemented with L-Glutamine, however it can be further supplemented according to user needs.

CELL RECOVERY AND ADAPTATION

1. Supplement IS Sf Insect medium (PN# 91174) as needed according to user preference. Prior to supplementation, note that the medium contains glutamine and Kolliphor® P188. Aseptically transfer appropriate volume (30 mL) of supplemented medium into an Optimum Growth 125 mL Flask (Thomson# 931110 or equivalent) and equilibrate in a 27ºC, 0% CO_2, 0% humidity incubator.
2. Thaw frozen cell vial rapidly in a 37°C water bath.
3. Cell Washing:
    a. Transfer cryovial contents into pre-equilibrated IS Sf Insect Media (PN# 91174) at a concentration of 2 x 10^6 cell/mL.
    b. Centrifuge conical tube at 200 x *g* for 5 minutes.
    c. Aseptically decant the supernatant.
4. Transfer the cells to a culture flask with pre-equilibrated IS Sf Insect medium (from step 1) to achieve an initial viable cell density of 3 x 10^6 cells/mL.
5. Incubate culture in a 27ºC, 0% CO_2, 0% humidity incubator for 3 to 4 days.
6. Following the *Subculturing Procedure* below, subculture cells for a minimum of three passages in IS Sf insect media.
7. To assure optimum performance and allow for complete adaptation, maintain cells in IS Sf Insect medium for several passages, and passage biweekly. A minimum of three passages in IS Sf Insect medium is strongly recommended before use in applications.
8. If severe cell aggregation is observed, continue passaging with the following recommendation:
    a. Supplement 2 mL/L Anti-Clumping Supplement (PN# 91150).
9. In most cases, Sf9 cells can be directly adapted into IS Sf Insect medium (PN# 91174). If cells grow slowly (less than 1 x 10^6 cells/mL within 4 days) with viability below 80%, continue passaging with the following recommendations:
    a. Increase seeding density to 1.5-2.0 x 10^6 cells/mL.
    b. Sequential adaption at ratios of 1:1, 1:2, 1:4, and 0:1 of current medium to IS Sf Insect.

SUBCULTURING PROCEDURE

Suspension culture conditions:

- Culture vessels: Optimum Growth 125 mL Flask (Thomson# 931110 or equivalent)
- Working volume: 20-75mL for 125mL flask
- Seeding density: 0.8 x10^6 cells/mL
- Incubator: 27°C, 0% CO_2 (ambient), non-humidified, 150rpm

1. Dispense appropriate volume (20-75mL) of IS Sf Insect medium into a culture vessel and equilibrate to 27°C (non-humidified).

2. Seed cells into culture vessel at 0.8 x10^6 viable cells/mL. It is important for the cells to be in logarithmic phase of growth with at least 90% viability before passaging. Following passaging, wait at least 15 minutes to count insect cultures.
3. Incubate cultures for 3-5 days or until the viable cell density reaches 8-10 x 10^6 viable cells/mL, at which point subculturing is required (repeat of the above recommended steps).

CRYOPRESERVATION

1. Prepare required volume of freezing medium (95% cold IS Sf Insect medium + 5% sterile DMSO). Keep at 4°C until ready to use.
2. Centrifuge appropriate number of healthy cells at 200 x g for 5 minutes. Decant or aspirate the supernatant without disturbing the cell pellet.
3. Re-suspend cells in cold freezing medium at a density of 2 x107 viable cells/mL (or desired cell density based on user needs)
4. Aliquot 1 mL/vial (or desired volume) into sterile cryovials.
5. Gradually lower the temperature of the vials to -80°C at a rate of -1°C/minute in an appropriate freezing container.
6. Once cells reach -80°C, transfer to liquid nitrogen vapor phase for long-term storage.

Example Data

HIGH DENSITY CULTURE

IS Sf Insect enables high viable cell density of Sf9 culture compared to commercially available media. Sf9 cells were cultured in Optimum Growth 125 mL Flask (Thomson #931110) at a working volume of 20mL per flask, and seeding density of 0.8 x 10^6 cells/mL in a 27°C, 0% CO_2, no humidity incubator. The rotation speed was set at 150 rpm.

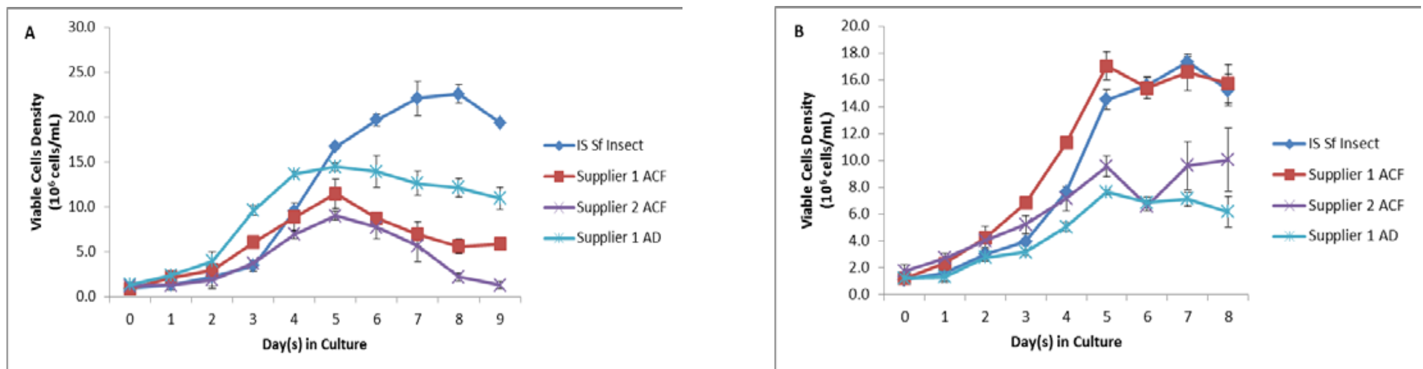

Figure 1. IS Sf Insect supports Sf9 and Sf21 high density cell cultures compared to other commercial serum-free insect cell media. Viable cell density (VCD) of Sf9 (A) and VCD of Sf21 (B) cells were measured for Sf9 and Sf21 cells cultured in IS Sf Insect, Supplier 1 animal component-free (ACF) or animal-derived (AD) media, or Supplier 2 ACF medium. Sf9 and Sf21 cells were cultured in Optimum Growth 125 mL Flask (Thomson #931110) at a working volume of 20mL per flask, and seeding density of 0.8 x 10^6 cells/mL for Sf9 and 1.0 x 10^6 cells/mL for Sf21 in a 27°C, 0% CO_2, no humidity incubator. The rotation speed was set at 150 rpm. Data shown represents the average of four replicate cultures.

TRANSIENT PROTEIN EXPRESSION

Since optimal transfection parameters may vary based on a number of factors (cell type, method of transfection and DNA source), it is highly encouraged that parameters are optimized prior to use based on user specifications. The following transient transfection protocol can be used as a general guideline to begin optimization work.

Recommended culture conditions:

- Cells: Sf9 or Sf21 cells
- Medium: IS Sf Insect
- Culture vessels: Thompson 125 mL polycarbonate shake flask
- Working Volume: 20-75 mL
- Seeding density: 0.8 x10^6 cells/mL
- Rotation speed: 150 rpm
- Incubator: 27°C, 0% CO_2, no humidity

1. Count insect cells using preferred cell counting equipment.
2. Follow the aforementioned sub-culturing directions to achieve a seeding density of 0.8 x10^6 cells/mL.
3. To assure optimum performance and allow for complete adaptation, maintain cells in IS Sf Insect medium for several passages, and passage biweekly. A minimum of three passages in IS Sf Insect medium is strongly recommended before use.
4. Prior to transfection, cells must be sub-cultured to an adequate density for achieving the desired MOI.
5. Transfect cells with the desired volume of viral stock.
6. Count cells 15 minutes after post-transfection incubation.
7. Incubate cells for 3 days after transfection, and measure cell density once every 24 hours.
8. On the third day post-transfection, analyze cultures for transient protein expression (this can be done by a variety of methods including, but not limited to ELISA, Western blot, fluorescence microscopy or flow cytometry).

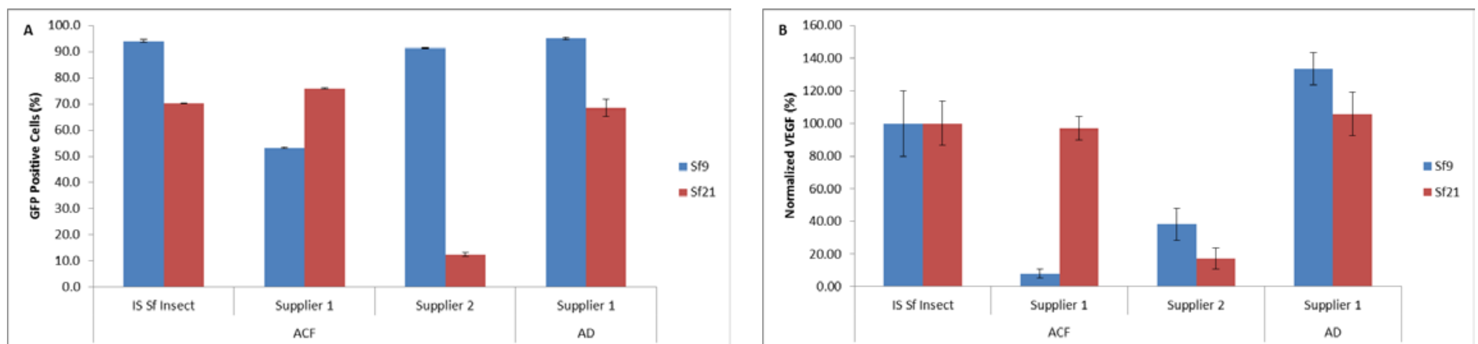

Figure 2. IS Sf Insect supports high infection rates by BEVS and protein yields compared to other commercial serum-free insect cell media. Sf9 and Sf21 cells were infected with BEVS expressing Vascular Endothelial Growth Factor (VEGF) on Day 0 (Sf9: MOI=0.1, Sf21: MOI=0.5). Infection efficiencies were measured by the percentage of cells expressing GFP (A) through flow cytometry on Day 3 post-infection. The protein yields were measured by VEGF ELISA (B), performed also on Day 3 post-infection. Data shown in B were normalized against the average VEGF value expressed in IS Sf Insect. Sf9 cells and Sf21 cells were cultured in Optimum Growth 125 mL Flask (Thomson #931110) at a working volume of 20mL per flask, and seeding density of 0.8 x 10^6 cells/mL (Sf9) and 1.0 x 10^6 cells/mL (Sf21) in a 27°C, 0% CO_2, no humidity incubator. The rotation speed was set at 150 rpm. Data shown above represent the average of four replicate cultures.

Related Products

Catalog #	Product	Size
91150	Anti-Clumping Supplement	50 mL

Technical Support

CONTACT US

For more information or assistance contact Customer Service at:

- Email: fisitmrequest@fujifilm.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- SDS
- COAs (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

FUJIFILM Irvine Scientific, Inc.
2511 Daimler Street, Santa Ana, California 92705-5588 USA
Telephone: 1 949 261 7800 • 1 800 437 5706
Fax: 1 949 261 6522 • www.irvinesci.com

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