

S2 (*Drosophila Schneider 2*) Insect Cells

INSTRUCTIONS FOR USE

Product Description

The S2 cell line was derived from a primary culture of *Drosophila melanogaster* embryos. S2 cells are adapted for suspension culture in ESF 921 and are available as a frozen vial or suspension culture.

For Research Use Only. Not for use in diagnostic procedures.

Product	Catalog Number	Amount	Storage
S2 cells adapted in ESF 921, frozen vial	94-005F	100 million cells per vial	Thaw immediately or LN ₂
S2 cells adapted in ESF 921, suspension culture	94-005S	50 million cells in 50 mL media	Culture immediately

Important Information

ESF 921 is a 1X complete, ready to use media. Do not add L-Glutamine or surfactants such as Pluronic® F-68. Antibiotics are not recommended; however, Penicillin-Streptomycin or Gentamicin may be used when required.

Safety Information

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

Culture Conditions

Media: ESF 921*

Cell Line(s): S2

Culture Type: Suspension or adherent

Recommended Culture Vessels: Shake flasks or T-Flasks

Temperature Range: 27°C to 28°C

Incubator Atmosphere: Non-humidified, non-CO₂ atmosphere. Ensure proper gas exchange and minimize exposure of cultures to light.

***Note:** S2 cell lines require Fetal Bovine Serum (FBS) during the recovery process after thaw. Use 5% FBS as directed below.

Receiving Frozen Cells

S2 cells are frozen in ESF 921 with 10% DMSO and 1% FBS. There are 100 x 10⁶ cells per vial.

1. Prepare for thawing cells by placing 50 mL of room temperature ESF 921 containing 5% FBS into a 125 mL Erlenmeyer shake flask.
2. Thaw frozen cells rapidly by swirling in a 37°C water bath. Thaw vial until a small amount of ice remains. DO NOT leave vial unattended.
3. Sanitize outer surface of vial with 70% alcohol. Transfer contents of vial to culture flask using a 2 mL pipette. DO NOT pour.
4. Incubate overnight at 27°C in a shaking incubator. Ensure caps (both vented and solid) are loosened to improve gas exchange. Determine count and viability. Cells should start doubling 24 hours after thaw. S2 cells are cryopreserved in the presence of FBS and can be weaned off FBS over the course of several passages.

Receiving Suspension Cultures

S2 cells are packaged in a 125 mL Erlenmeyer shake flask. There are 50 x 10⁶ cells per flask, 1 x 10⁶ cells/ml in 50 ml of ESF 921.

1. Remove parafilm and loosen cap to allow for gas exchange. Determine cell count and viability.
2. Place flask in a shaking incubator at 130-135 rpm at 27°C. Cells should start doubling a day after receipt. It is not unusual for the cell count to remain the same for the first 24 hours following receipt.

Suspension Cell Culture

Sf9			
Max Density	>50x10 ⁶ /mL	Split Density	25-35x10 ⁶ /mL
Seed Density	2-5x10 ⁶ /mL	Split Frequency	2x/week

Passage the cells twice a week on a Mon/Thurs or Tues/Fri schedule. Repeatedly allowing the cells to reach maximum density may change the growth kinetics of the culture. Split the cells while still in mid-log phase growth.

Note: It is recommended that a growth curve be determined using the user's standard culturing conditions. This will allow for determination of mid-log phase growth.

1. Determine viable cell count.
2. Seed shake flask at a density shown above. Use 30-50 mL for a 125 mL Erlenmeyer shake flask.
3. Incubate at 27°C in a non-humidified, non-CO₂ atmosphere incubator. Rotate shake flask cultures on an orbital shaker platform at 130-135 rpm. Loosen caps (both solid and vented) to allow for gas exchange.
4. Passage when viable cells density reaches 25-35 x10⁶ cells per mL.
5. It is recommended to thaw a new vial of cells every 3 months. Cultures may be maintained for longer but increase the risk of accumulating environmental stresses that can impact the growth and performance characteristics of the culture.

Adherent Cell Culture

1. Observe cell monolayer using an inverted microscope to ensure confluence. Remove media and any floating cells using a sterile pipette or by aspiration.
2. Add 4 mL (per 25 cm²) ESF 921 to the flask and resuspend the cells by repeatedly pipetting the medium across the monolayer. It may be necessary to aid cell detachment by tapping the side of the flask against a hard surface.
3. Determine the viable cell density of the cell suspension.
4. Inoculate 1-2 x 10⁶ cells (per 25 cm²) into new culture flasks containing room temperature ESF 921 (5 mL per 25 cm²).
5. Incubate at 27°C in a non-humidified, non-CO₂ atmosphere incubator. Ensure air exchange by loosening caps. Vented caps are recommended.

Cryopreservation

1. Freezing medium is sterile filtered 89% ESF 921, 1% FBS plus 10% DMSO. 0.15 M trehalose may be added. Store and use at 4°C.
2. Prepare the desired quantity of cells, harvesting in mid-log growth with viability >90%.
3. Determine the viable cell density and calculate the required volume of freezing medium to give a final cell density between 50-100 x 10⁶ cells/mL.
4. Harvest the cells by centrifugation at 1000 rpm for 5 minutes. Resuspend the cells in the pre-determined volume of 4°C freezing medium.
5. Dispense 1 mL aliquots of suspension into cryovials.
6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen, we recommend vapor phase storage at -200 °C to -125 °C.








Limited Product Warranty

Expression Systems LLC warrants that this product meets its specifications, as stated in our product brochures and certificates. This warranty lasts from the time we deliver the consumable until either the consumable's shelf life, when the product has been handled and stored in accordance with this IFU.

Related Products

Product	Catalog Number
ESF 921	96-001
ESF AF	99-300
Production Boost Additive	95-006
Adapted Sf9 Cells	94-001 or 94-006
Adapted Sf21 Cells	94-003 or 94-010
Adapted Tni Cells	94-002 or 94-011
Adapted S2 Cells	94-005 or 94-012
Transfection Medium	95-020

Legend of Labeling Symbols

Symbol	Interpretation
	Catalog Number
	Lot Number
	<i>Research Use Only</i>
	Manufacturer
	Temperature Limitation
	Date of Manufacture
	Instruction for Use

Important Licensing Information

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For technical assistance or documentation, such as Certificates of Analysis or Safety Data Sheets, email support@expressionsystems.com

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