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	50 million cells per vial	Thaw immediately or LN_2
S2 cells adapted in ESF 921, suspension culture	94-005S	100 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 spinner bottle
Temperature Range: 27°C to 28°C
Incubator Atmosphere: Non-humidified, non-CO_2 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 10^6 cells per vial. Prepare for thawing cells by placing 50 ml of room temperature ESF 921 containing 5% FBS into a 125 ml Erlenmeyer shake flask. Thaw frozen cells rapidly by shaking in a 37ºC water bath. Thaw vial until a small amount of ice remains. DO NOT leave vial unattended. Transfer contents of vial to culture flask using a 1 ml pipette. DO NOT pour. Incubate overnight at 27ºC in a shaking incubator. 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 of 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 10^6 cells per flask, 1 x 10^6 cells/ml in 50 ml of ESF 921. Remove parafilm and loosen cap for good aeration. Place flask in a shaking incubator at 120-140 rpm at 27ºC. Determine count and viability. 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

	S2
Max Density	>50 x 10^6/mL
Split Density	25-35 x 10^6/mL
Seed Density	2-5 x 10^6/mL
Split Frequency	2-3x/week

It is recommended to passage the cells three days a week on a Mon/Wed/Fri schedule or twice a week on a Mon/Thurs or Tues/Fri schedule. It is not advised to repeatedly allow the cells to reach maximum densities as the growth kinetics of the culture may change. Try to keep the maximum cell density to mid-log phase.

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, 50-75 mL for 100 mL spinner bottle.
3. Incubate at 27°C in a non-humidified, non-CO_2 atmosphere incubator. Rotate shake flask cultures on an orbital shaker platform at 120-140 rpm. Loosen caps to allow for gas exchange. For spinner cultures, set impeller stirring rate to 85-95 rpm (rpm may vary with impeller design). Loosen side arm caps to allow for gas exchange.
4. Passage when viable cells density reaches 25-35 x10^6 cells/mL.
5. It is recommended to thaw a new vial of cells every 3 months. Cultures may be maintained for a longer time period 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 cm^2) 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 10^6 cells (per 25 cm^2) into new culture flasks containing room temperature ESF 921 (5 mL per 25 cm^2).
5. Incubate at 27°C in a non-humidified, non-CO_2 atmosphere incubator. Loosen caps or use flasks with vented caps (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 4ºC.
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 10^6 cells/mL.
4. Harvest the cells by centrifugation at 1000 rpm for 5 minutes. Resuspend the cells in the pre-determined volume of 4ºC 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 (1ºC decrease per minute).
7. Transfer frozen cells to liquid nitrogen, we recommend vapor phase storage at -200 ºC to -125 ºC.

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
REF	Catalog Number
LOT	Lot Number
RUO	*Research Use Only*
	Manufacturer
	Temperature Limitation
	Date of Manufacture
	Instruction for Use

Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

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.

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

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