

### Product Description

ESF 921™ Insect Cell Culture Medium is a complete serum-free, protein-free medium developed for robust cell growth, protein expression and baculovirus vector production for a wide range of insect cells including Sf9, Sf21, Tni, and Drosophila S2. ESF 921 contains L-Glutamine and Kolliphor® P188 (Pluronic® F-68). For Research Use Only. Not for use in diagnostic procedures.

Product	Catalog Number	Volume	Storage	Shelf Life
ESF 921 (1X), liquid	96-001-01	1 Liter	2°C to 8°C, protected from light	12 months from Date of Manufacture
	96-001-08	8 Liter, Media Transfer Bag		
	96-001-10	10 Liter, Media Transfer Bag		
	96-001-20	20 Liter, Media Transfer Bag		
	96-001-50	50 Liter, Media Transfer Bag in Drum		
	Custom	Inquire for Custom Fill Volumes		

### Important Information

ESF 921 is a 1X complete, ready to use medium. 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):** Sf9, Sf21, Tni, 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<sub>2</sub> atmosphere. Ensure proper gas exchange and minimize exposure of cultures to light.

### Suspension Cell Culture

	Sf9	Sf21	Tni	S2
Max Density	>15 x10 <sup>6</sup> /mL	>5 x 10 <sup>6</sup> /mL	>8 x 10 <sup>6</sup> /mL	>100 x 10 <sup>6</sup> /mL
Split Density	6-8 x 10 <sup>6</sup> /mL	4-5 x 10 <sup>6</sup> /mL	6-7 x 10 <sup>6</sup> /mL	25-75 x 10 <sup>6</sup> /mL
Seed Density	0.75-1 x 10 <sup>6</sup> /mL	0.5-1 x 10 <sup>6</sup> /mL	0.5-1 x 10 <sup>6</sup> /mL	2-5 x 10 <sup>6</sup> /mL
Split Frequency	2- 3x/week	2- 3x/week	2- 3x/week	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 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.*

- Determine viable cell count.
- 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.

- Incubate at 27°C in a non-humidified, non-CO<sub>2</sub> 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.
- Passage when viable cells density reaches 6-8 x 10<sup>6</sup> cells per mL.
- 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.

### Monolayer Cell Culture

- Observe cell monolayer using an inverted microscope to ensure confluence. Remove media and any floating cells using a sterile pipette or by aspiration.
- Add 4 mL (per 25 cm<sup>2</sup>) 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.
- Determine the viable cell density of the cell suspension.
- Inoculate 0.5-1 x 10<sup>6</sup> cells (per 25 cm<sup>2</sup>) into new culture flasks containing room temperature ESF 921 (5 mL per 25 cm<sup>2</sup>).
- Incubate at 27°C in a non-humidified, non-CO<sub>2</sub> atmosphere incubator. Loosen caps or use flasks with vented caps (recommended).

### Adaptation of Cells to ESF 921

It is recommended to use sequential adaptation when adapting cells to ESF 921 medium. It is critical that cell viability be at least 95% (Sf9), 93% (Tni), 90% (Sf21) or 85% (S2), and the growth rate be in mid-logarithmic phase prior to initiating the adaptation process.

- Passage cells into a 50:50 ratio of ESF 921 to the original media. During the adaptation process, use a seeding density of 1 x 10<sup>6</sup> cells per mL.
- Incubate at 27°C in a non-humidified, non-CO<sub>2</sub> atmosphere incubator. Rotate shake flask cultures on an orbital shaker platform at 120-140 rpm. Loosen caps to allow for gas exchange.
- Passage when the viable cell count is 4-6 x 10<sup>6</sup> cells per mL (3-4 days post-split). Passage into a 75:25 ratio of ESF 921 to original medium.

- Repeat previous step, increasing the ratio of ESF 921 to original medium (75:25 followed by 88:12, 95:5) until the cells are in 100% ESF 921. It may be necessary to perform multiple passages in one ratio format.
- Insect cells may swell during adaptation. An increase of 2-3 microns is normal, an increase of 5 microns or more is suggestive of a stressed culture and the adaptation should be started again with fresh cells.

After several passages in 100% ESF 921, the viable cell count should conform to the table above with viabilities > 90%.








#### Cryopreservation

- Freezing medium is sterile filtered 90% ESF 921 plus 10% DMSO. 0.15 M trehalose may be added. Store and use at 4°C.
- Prepare the desired quantity of cells, harvesting in mid-log growth with viability >90%.
- Determine the viable cell density and calculate the required volume of freezing medium to give a final cell density between 25-50 x 10<sup>6</sup> cells per mL.
- Harvest the cells by centrifugation at 1000 rpm for 5 minutes. Resuspend the cells in the pre-determined volume of 4°C freezing medium.
- Dispense 1 mL aliquots of suspension into cryovials.
- Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
- Transfer frozen cells to liquid nitrogen, we recommend vapor phase storage at -200 °C to -125 °C.

#### Related Products

Product	Catalog Number
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
BestBac™ Linearized DNA	91-001 or 91-002
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

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@expressionssystem.com](mailto:support@expressionssystem.com)

©2016 Expression Systems LLC. All rights reserved.

Kolliphor® (Pluronic® F-68) is a registered trademark of BASF Corporation.

DISCLAIMER – EXPRESSION SYSTEMS LLC DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL EXPRESSION SYSTEMS AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

