TniPRO™ in ESF AdvanCD™ Insect Cells
INSTRUCTIONS FOR USE

Product Description
TniPRO™ cells are ovarian cells isolated from *Trichoplusia ni.* TniPRO cells were isolated in ESF AF and have never seen exogenous animal proteins. TniPRO™ cells are adapted for suspension culture in ESF AdvanCD™ and are available as a frozen vial.
For Research Use Only. Not for use in diagnostic procedures.

Product	Catalog Number	Amount	Storage
Tni PRO™ cells in ESF AdvanCD™	94-031	50 million cells per vial	Thaw immediately or LN_2

Important Information
ESF AdvanCD is 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 AdvanCD

Cell Line(s): Tni PRO™

Culture Type: Suspension

Recommended Culture Vessels: Shake flasks

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.

Receiving Frozen Cells
Insect cells are frozen in ESF AdvanCD with 10% DMSO. There are 50 x 10^6 cells per vial.

1. Prepare for thawing cells by placing 50 mL of room temperature ESF AdvanCD into a 125 mL Erlenmeyer shake flask.
2. Thaw frozen cells rapidly by swirling in a 37ºC 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 27ºC in a non-humidified, non-CO_2 atmosphere incubator. Loosen caps (both solid and vented) to allow for gas exchange. Allow the cells to achieve a density of at least 4 x 10^6 cells per mL before passaging.
5. Split cells at a density of 1 x 10^6 cells per mL for the first week after thaw. Follow directions for suspension culture thereafter.

Suspension Cell Culture

Tni PRO™			
Max Density	>8x10^6/mL	Split Density	6-7x10^6/mL
Seed Density	0.3-1x10^6/mL	Split Frequency	2x or 3x/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-CO_2 atmosphere incubator. Shake 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 6-7 x10^6 cells/mL. If passaging 3 times per week, split to 0.5x10^6/mL. If passaging 2 times per week, split to 0.5x10^6/mL on Monday and 0.3x10^6/mL on Thursday.
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.

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Cryopreservation

1. Freezing medium is sterile filtered 90% ESF AdvanCD 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 25-50 x 10^6 cells/mL.
4. Harvest the cells by centrifugation at 300-400 x g 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 AdvanCD™	54-018
Virus Stabilization Additive	95-010
BestBac™ Linearized DNA	91-001 or 91-002
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

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.

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.

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

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