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Growth and maintenance of Mimic™ insect cells
USER GUIDE

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ThermoFisher SCIENTIFIC

Manufacturer's address: Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, CA 92008
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Revision history: Pub. No. MAN0000298

Revision	Date	Description
A.0	14 March 2017	Rebrand and update legal boilerplates
03 January 2012	03 January 2012	Baseline for this revision

Contents

Product information

Introduction

Mimic™ Sf9 cells are prepared from Master Cell Bank cultures and shipped at less than 30 total passages. Mimic™ cells are transgenic insect cells that have been engineered to produce recombinant proteins with terminally sialylated N-glycans like those found in mammalian systems. These are identical to the SfSWT-1 cells developed by Donald L. Jarvis. This cell line is suitable for expressing recombinant glycoproteins with baculovirus and other insect expression systems, including the Bac-to-Bac™ Expression System and the InsectSelect™ System.

Contents and storage

Mimic™ Sf9 Insect Cells (Cat. No. 12552014)

Contents	Amount	Storage[1]
Mimic™ Sf9 Insect Cells, 1×10^7 cells[2]	1 mL	Liquid nitrogen

[1] Cells are shipped on dry ice

[2] Cells are supplied in 60% Grace's Media, 30% FBS, and 10% DMSO

Overview of Mimic™ cells

Characteristics of Mimic™ Insect Cells

N-Glycosylation: Insect cells, like other eukaryotic cells, modify many of their proteins by N-glycosylation. However, endogenous insect cell N-glycan processing generally does not produce complex, terminally sialylated N-glycans like those produced by mammalian cells.

Mimic™ cell lines have been stably integrated with the following mammalian glycosyltransferases:

- α2,6-sialyltransferase
- α2,3-sialyltransferase (expressed, but not active)
- β4-galactosyltransferase
- N-acetylglucosaminyltransferase I
- N-acetylglucosaminyltransferase II

These enzymes result in the production of highly processed, mammalian-like recombinant glycoproteins with biantennary, sialylated, complex glycans.

Origin: Mimic™ cells are derived from the Sf9 cell line, which are a traditional cell line used with baculovirus. Sf9 cells originated from the IPLBSF-21 cell line, derived from the pupal ovarian tissue of the fall army worm, *Spodoptera frugiperda*.

Growth characteristics: Mimic™ cells grow well in adherent and suspension culture. Media with serum is required. Doubling times for Mimic™ cells are similar to those for Sf9 cells.

Appearance: Mimic™ cells are spherical with some granular appearance.

Uses: Mimic™ cells lines are suitable for transfection and expression of recombinant proteins.

Insect expression systems

A number of insect expression systems are available to facilitate expression of recombinant proteins in Mimic™ cells. The Bac-to-Bac™ Baculovirus Expression System allows recombinant protein expression using baculovirus, while the InsectSelect™ System allows protein expression using a non-lytic, stable system. For more information about these expression systems, refer to **thermofisher.com** or contact Technical Support (see page 26).

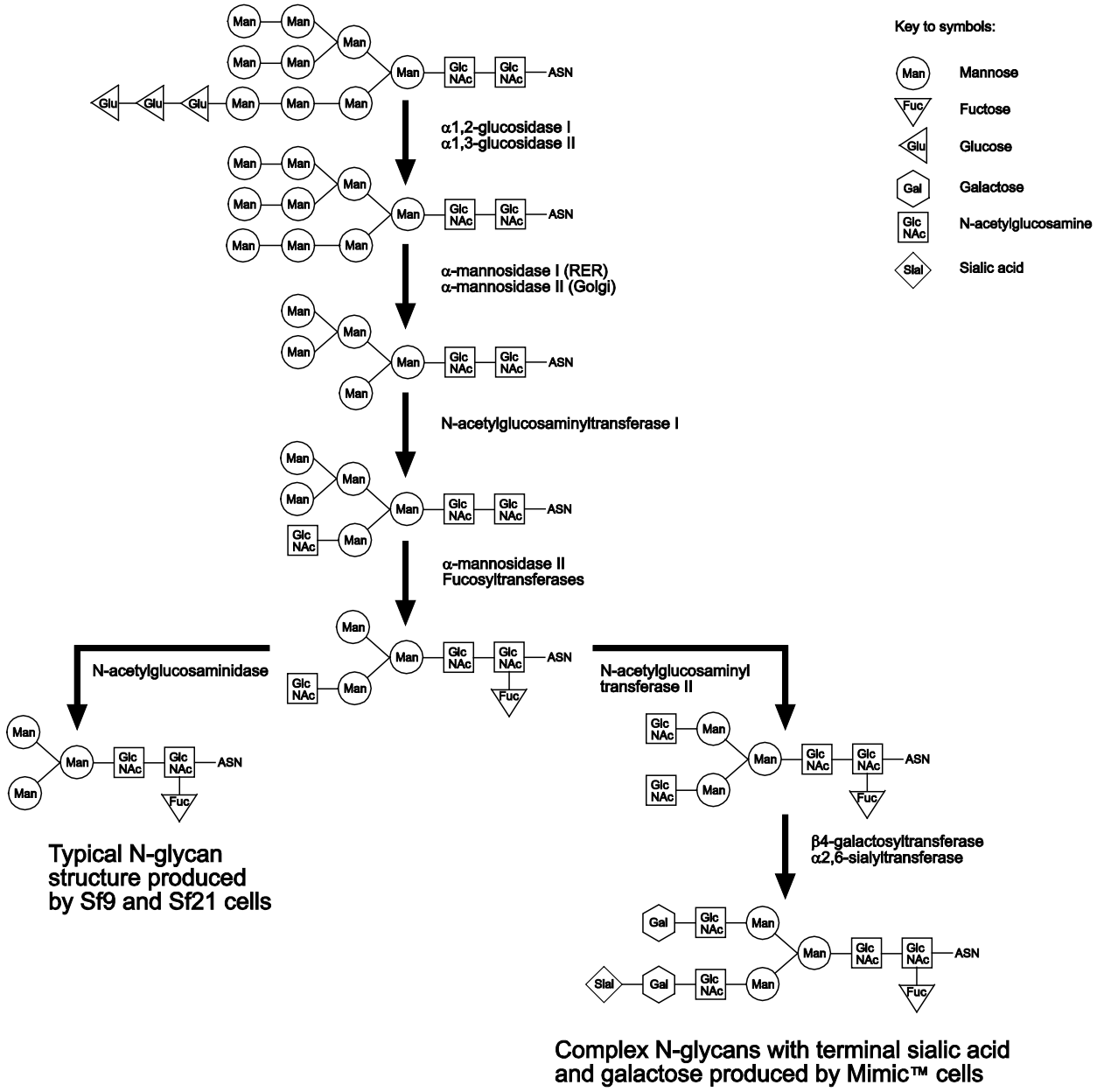
Compatibility

Mimic™ cells are not compatible with Bac-N-Blue™ Baculovirus Expression System. Bac-N-Blue™ viruses constitutively express active β-galactosidase, which will remove galactose from the N-glycan side chains of recombinant proteins expressed by Mimic™ cells.

Overview of Mimic™ insect cells, continued

N-Glycosylation pathway

The figure below provides a model of insect cell and Mimic™ cell N-glycosylation pathways. Mimic™ cells have been modified to greatly enhance the production of complex, sialylated N-linked glycans, as shown in the lower right branch of the pathway.



Media

Complete TNM-FH

Mimic™ cells require complete TNM-FH (*Trichoplusia ni* Medium-Formulation Hink) for growth. TNM-FH is Grace's Insect Medium, Supplemented, plus 10% fetal bovine serum (see page 25 for ordering information). Serum provides a source of sialic acid and additional nutrients.

Grace's Insect Medium, Supplemented, includes TC yeastolate, lactalbumin hydrolysate, and L-glutamine.

When preparing complete TNM-FH, keep in mind the following:

- **Serum Sensitivity:** Serum does not need to be heat-inactivated prior to use. However, the quality of serum is important for optimal cell growth. We strongly recommend that you test a small aliquot of each new lot on your cells before using it. For more information on serum sensitivity, see **Troubleshooting**, page 20.
- **pH:** TNM-FH does not contain pH indicators. The normal pH for Mimic™ cells in this medium is 6.2. Unlike mammalian cell cultures, the pH rises gradually as the cells grow, but usually does not exceed pH 6.4.
- **Stability:** Complete TNM-FH medium is stable for 1 month at 4°C.

Important Grace's Medium will not support the growth of Mimic™ cells without the additional supplements and serum.

Antibiotics

The use of antibiotics is optional. Many antibiotics are suitable for use with Mimic™ cells. The following table summarizes some of the most common.

Antibiotic	Working conc.	Method of action
Gentamycin	10 µg/mL	Inhibits bacterial protein synthesis
Amphotericin B (Fungizone)	0.25 µg/mL	Binds sterols and interferes with membrane permeability
Penicillin-Streptomycin	100-200 U/mL 100 µg/mL	Inhibits bacterial cell wall synthesis Inhibits bacterial protein synthesis

Prepare complete TNM-FH Medium

If possible, prepare complete TNM-FH medium just prior to use. The medium may be stored at 4°C for up to 1 month if it does not become contaminated.

1. Add 55 mL of FBS to 500 mL of Grace's Insect Medium, Supplemented. If desired, add antibiotic at the appropriate concentration.
2. Filter this solution through a 0.2-µm filter into a sterile container.
3. Store complete TNM-FH medium at 4°C.
4. Equilibrate complete TNM-FH to room temperature before use.

Baculovirus and media

When working with recombinant or wild-type viral stocks, always maintain separate media bottles for cell culture and for virus work. Baculovirus particles can survive and be maintained in media at 4°C and will contaminate your cell cultures if added to plates or flask during passaging.

Methods

Maintain and handle cells

Sterile technique

All handling of Mimic™ cells should be performed in a laminar flow hood, using sterile conditions and equipment.

CO_2

CO_2 exchange is not required for these cells.

Growth temperature

Fresh cell culture medium should be equilibrated to room temperature before use. Cells can be maintained at room temperature on the bench top or in a drawer; however, a 27°C controlled environment is recommended.

Below 27°C, Mimic™ cells will grow more slowly, but will recover and resume normal doubling times once the temperature is returned to 27°C. Above 30°C, cells may display decreased viabilities. Cells under prolonged exposure to temperatures above 30°C should not be used.

Confluency

Confluency is a marker for when to subculture your cells. A confluent monolayer is an adherent cell culture (dish, plate or flask) in which the cells have formed a single layer over the entire surface area available for growth.

Floaters

Floaters are cells that are either loosely attached or suspended in the medium. Floaters are a normal occurrence, and are not necessarily an indicator of cell mortality.

To check the viability of floaters, remove a small aliquot of medium containing floaters and assay for cell viability using the Countess™ Automated Cell Counter or trypan blue exlusion method.(see protocol on page 18). If the viability is high (>95%), the cells may be propagated by transferring the medium containing floaters to a new, appropriately sized flask containing fresh medium.

Cell counter

Countess™ Automated Cell Counter is a benchtop counter designed to measure cell count and viability (live, dead, and total cells) accurately and precisely in less than a minute per sample, using the standard Trypan Blue technique (see page 25 for ordering information).

Using the same amount of sample that you currently use with the hemacytometer, the Countess™ Automated Cell Counter takes less than a minute per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells and provides information on cell size.

Continued on next page

Determine cell density and viability

Follow the procedure below to determine total cell count and viability of cells in a culture:

1. Transfer a small aliquot of the cell suspension culture to a microcentrifuge tube.
2. Determine the cell density electronically using a Countess™ Automated Cell Counter or a Coulter Counter, or manually using a hemacytometer chamber (see page 17).
3. Determine cell viability using the Countess™ Automated Cell Counter or the trypan blue exclusion method (see page 18 for procedure).

Cell viability should be regularly assessed during cell counts in order to maintain optimal adherent and suspension cultures.

Minimum viability requirement

Cell viability should be at least 95% for healthy log-phase cultures. Cells below 95% viability are not growing under optimal conditions and should not be used in experiments. See **Troubleshooting**, page 20 if your cell viability is below 95%.

Concentrate cells

Cells can be concentrated if their density is too low (less than 5×10^5 cells/mL) to support log phase growth. Concentrating cells to a higher density (1×10^6 cells/mL) will induce log phase growth (see page 10).

Cell log

We recommend keeping a record of cell density, viability, passage number, and other information as a decision-making and troubleshooting tool. See page 19 for a sample log form.

Remove and add medium—Monolayers in flasks

To **remove** medium from a flask of cells, tilt the flask so that all of the medium flows to one corner, away from the cell monolayer, and remove the medium carefully with a pipette. Avoid touching the cell monolayer.

To **add** medium to a flask: Gently pipet the medium down the side of the flask away from the cell monolayer.

Remove and add medium—Monolayers in plates

To **remove** medium from a plate containing a cell monolayer, tilt the plate at a 45°C angle so that the medium flows to one edge and aspirate the medium completely and very carefully using a Pasteur pipette.

To **add** medium to a plate, carefully and slowly add the medium against the side edge of the plate. Allow the medium to cover the entire surface of the plate, being careful not to dislodge cells.

Continued on next page

Remove and add medium—Suspension cultures

Remove medium from a shake flask or spinner:

1. Remove the screw cap from one arm of the flask.
2. Carefully insert a pipette without touching the flask and remove medium from the culture.
3. Carefully remove pipette from the arm of the flask without touching the sides or dropping any medium.

Change pipettes each time one is inserted into the flask and then removed.

Add medium to a shake flask or spinner:

1. Remove the screw cap from one arm of the flask.
2. Carefully insert pipette without touching the flask and add medium.
3. Carefully remove pipette from the arm of the flask without touching the sides or dropping any medium.

Change pipettes each time one is inserted into the flask and then removed.

Concentrate cells

To concentrate cells from a suspension culture (or resuspended cells from monolayer culture):

1. Transfer cell suspension to a sterile centrifuge tube of appropriate size and centrifuge for 5 minutes at 800 × g.

   Important: Do not centrifuge at higher speeds. Mimic™ cells are very sensitive to centrifugal force.
2. Carefully remove the supernatant without disturbing the pellet of cells.
3. Add the desired volume of fresh, complete TNM-FH gently to the side of the tube and slowly pipette up and down 2 to 3 times to resuspend the cell pellet.
4. Transfer to the desired, sterile container.

Disperse cells

Prior to performing transfections and plaque assays, cells need to be evenly distributed over the surface of a tissue culture plate. This ensures that:

- Cells do not distribute unevenly, leading to asymmetric monolayers.
- Maximum cell surface area is available for infection.

Procedure: To disperse cells, rock the flask or plate slowly by hand forward and backward, then side to side. Do this four times, watching carefully to be sure the liquid reaches all areas of the growth surface. Do **not** use a circular motion to disperse cells as this causes a concentration of cells around the edges of the plate rather than an even distribution.

Initiate cell culture from frozen stock

Introduction

Use the following protocols to initiate a culture from a frozen stock of Mimic™ cells.

Required materials

- **Frozen Mimic™ cells**, in vials
- **Water bath** at 37°C
- **T-25 cm² flask** (per vial of cells)
- **Complete TNM-FH** (see page 7)
- **27°C constant-temperature incubator**

Sterile technique

Handle Mimic™ cells under sterile conditions in a laminar flow hood.

Experimental outline

The table below outlines the steps to initiate a cell culture from frozen stock.

Step	Action
1	Remove cells from liquid nitrogen or dry ice (if just received)
2	Thaw cells into complete TNM-FH
3	Let cells attach for 30–45 minutes
4	Remove medium (with DMSO) and add fresh medium
5	Grow to confluency and passage
6	Subculture until cells are doubling every 24–30 hours and are 95% viable
7	Freeze down several vials of low passage cells as backup

Continued on next page

Initiate cell culture from frozen stock, continued

Initiate culture with frozen cells

Thawed Mimic™ cells do not always appear round; some may be amorphous or have a wrinkled appearance. A significant portion of the cells may have lysed. The cellular debris will be eliminated through successive rounds of subculturing.

The freezing process can damage cells, resulting in a 20–25% mortality rate upon thawing. For example, if 1×10^7 cells are frozen, 7.5×10^6–8×10^6 cells may be recovered.

1. Remove a vial of cells from dry ice or liquid nitrogen (if they were stored) and thaw rapidly in a 37°C water bath.
2. Remove from water bath and quickly decontaminate the outside of the vial by treating with 70% ethanol.
3. Transfer the 1 mL of cell suspension directly into a T-25 cm^2 flask containing 4 mL of complete TNM-FH.
4. Transfer flask to a 27°C incubator and allow cells to attach for 30–45 minutes.
5. After the cells are attached, gently remove the medium (see page 9). This must be done as soon as the cells have attached to remove the DMSO from the freezing medium. This step will also remove cellular debris and unhealthy cells that do not adhere.
6. Feed cells with 5 mL of fresh TNM-FH.
7. After 24 hours, change the medium. Continue to incubate until cells have formed a confluent monolayer.

At this point, cells should be subcultured in adherent culture (see Subculture cells in adherent culture, see page 13). Mimic™ cells should be established in adherent culture before scaling up into suspension culture.

Subculture cells in adherent culture

Introduction

This section describes how to subculture Mimic™ cells in adherent culture. Mimic™ cells should be established in adherent culture before scaling up into suspension culture.

When to subculture

Adherent cultures should be passaged at >80 percent confluency.

IMPORTANT: Do not overgrow cells. Repeated subculturing of cells at densities past confluency will result in decreased doubling times and decreased viabilities.

We recommend thawing a fresh, low-passage culture of frozen cells every 3 months or 30 passages. We recommend keeping a log of the cell density, viability, passage number, and appearance. See page 19 for a sample cell log.

Required materials

- **T-flasks** of desired size
- **Complete TNM-FH** (see recipe on page 7)
- **Sterile pipettes**

Recommended dilution and culture volumes

Dilute Mimic™ cells at no greater than a 1:2 to 1:3 dilution (volume of cells: final volume of medium) to maintain log phase growth.

Do not split cells back too far. Densities lower than 33% confluency will inhibit growth.

The table below summarizes recommended flask volumes for adherent culture.

Flask size	Volume range
T-25 cm^2	5–10 mL
T-75 cm^2	15–20 mL
T-150 cm^2	40–50 mL

Subculture procedure

We recommend sloughing to dislodge monolayers in adherent culture. Follow the procedure below to subculture adherent cultures:

1. Remove all but 5 mL of medium from flask (see page 9).
2. Tilt flask on end so that the remaining medium flows to one corner, away from the cells.
3. Draw up some of the remaining medium into a sterile Pasteur pipette and, starting at the bottom corner of the flask, stream medium across the cells (sloughing). Dislodge cells using side-to-side gentle streaming motion as you move from the bottom corner up to the top opposite corner of the flask.
4. Determine cell density and viability (see protocols on pages 17–18).
5. Seed cells at a 1:2 to 1:3 dilution in a T-flask (see sizes and volumes above) for adherent culture, or transfer to suspension culture at a density of 1×10^6 cells/mL (see page Subculture cells in suspension culture, page 14).

Subculture cells in suspension culture

Required materials

- **Adherent Mimic™ cells.** Cells must be grown to seed a shake flask at a minimum density of 1×10^6 cells per mL.
- **27°C constant-temperature incubator**; CO_2 not required.
- **Rotating, orbital shaker platform**.
- **Disposable, sterile Erlenmeyer flasks.** Glass flasks without baffles may be used, but make sure to clean flasks thoroughly after each use to avoid potential toxicity.
- **Complete TNM-FH containing Pluronic™ F-68** at 0.1% final concentration.

Important

Mimic™ cells should be established in adherent culture before scaling up into suspension culture.

Recommended seeding density and culture volumes

Suspension cultures should be seeded at a density of 1×10^6 cells/mL.

For shaker cultures, we recommend the flask sizes and total working volumes below:

Flask size	Volume range
125 mL	35–50 mL
250 mL	75–100 mL

Pluronic™ F-68

For suspension cultures, Pluronic™ F-68 (Catalog No. 24040-032) should be added to your complete TNM-FH medium at a final concentration of 0.1%.

Establish shake flask cultures

To initiate a suspension culture of Mimic™ cells in shake flasks:

1. Grow enough log phase adherent cells to start a shake flask of desired size with 1×10^6 cells/mL. Determine the density and viability of adherent cells according to the protocols on pages 17–18. Seeding at lower densities can cause decreased doubling times in the initial stages of culture.

   Example: A 125-mL shake flask with 35 mL of culture at 1×10^6 cells/mL would require 3.5×10^7 viable cells.
2. Seed a clean, sterile shake flask with cells at a density of 1×10^6 cells/mL. Use fresh, complete TNM-FH with 0.1% Pluronic™ F-68.
3. Loosen flask caps to allow for aeration.
4. Incubate flask at 27°C on a rotating orbital shaker at 85–95 rpm. Check cells daily for density and viability.

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Subculture suspension cell cultures, continued

Subculture shake flask cultures

Passage cells in suspension culture when they reach a density of 2.0×10^6 to 2.5×10^6 cells/mL.

Example: A 125-mL shake flask containing 50 mL of cell culture is at a density of 2×10^6 cells/mL. Remove 25 mL of medium and cells and seed in a new flask with 25 mL of fresh complete medium for a final density of 1×10^6 cells/mL.

Maintain shake flask cultures

- **Renew cultures every month:** Cells can be kept in suspension for up to one month in log phase. At this point, a new culture should be initiated as cells begin to lose their infectivity and are no longer optimal.
- **Maintain log phase cell density:** Maintain density above 1×10^6 cells/mL for log phase growth. When cells reach a density of 2.0×10^6 to 2.5×10^6 cells/mL, dilute them with fresh medium to 1×10^6 cells/mL.
- **Maintain volumes for adequate aeration:** The total culture volume in a shake flask should not exceed 40% of the indicated volume of the flask for proper aeration (e.g., a 250-mL flask should not contain more than 100 mL of culture).
- **To reduce accumulation of cell debris and metabolic waste by-products,** we recommend gently centrifuging the cell suspension at $100 \times g$ for 5 minutes and resuspending the cell pellet in fresh, complete TNM-FH once every 3 weeks (see page 10 for detailed instructions on concentrating cells).
- **Keep a log:** We recommend keeping a log of the cell density, viability, passage number, and appearance. See page 19 for a sample cell log.

Spinner cultures

Follow the guidelines below to scale up from shake flasks to spinner cultures.

- **Flask:** We recommend starting with 100-mL or 250-mL spinner flasks, as these require fewer total cells than larger flasks to initiate culture.
- **Seeding density:** 1×10^6 cells per mL.
- **Spinner culture volume:** To ensure proper aeration:
  - The **maximum** culture volume should not exceed 50% of the spinner volume (e.g., a 250-mL spinner should have a culture volume of ≤125 mL).
  - The **minimum** culture volume should submerge the impeller to a depth of at least 1 cm (e.g., a 250-mL spinner should have a culture volume of ≥80 mL).
- **Impeller:** We recommend using a spinner flask with a **vertical impeller** rather than one with a hanging stir-bar assembly, because a vertical impeller provides better aeration. Incubate at 27°C with an impeller speed of 80–95 rpm.
- **Spinner caps:** Finger-tighten caps. Loose caps are not necessary for aeration.
- **Subculturing:** Subculture the cells when their density reaches 2.0×10^6 to 2.5×10^6 cells/mL. Maintain density above 1×10^6 cells/mL for log phase growth.
- **Cleaning:** Do not use soap when cleaning spinners, as residue will cause cell mortality. See page 18 for more information.

Freeze cells

Introduction

Once Mimic™ cell lines are established and doubling regularly, they can be frozen. Cells should be at least 90% viable and in mid-log exponential growth. You will need enough cells to freeze down 2–4 cryovials at a density of 1×10^7 each. We recommend that you freeze down several vials as soon as your cell culture meets these requirements (e.g., at as low a passage number as possible).

Freezing Procedure

To make the freezing medium, combine:

60% Grace's Insect Medium, Unsupplemented
30% FBS
10% DMSO

Note that Grace's Insect Medium does not contain supplements or FBS.

1. Count cells using a hemacytometer, as described on page 17. You need enough cells to freeze down 2–4 cryovials at a density of 1×10^7 each. Cells can come from suspension or adherent culture.
2. Set up sterile cryovials on ice. Be sure to label the cryovials.
3. Centrifuge the cells at 400–600 × g for 10 minutes at room temperature. Remove the supernatant.
4. Resuspend cells to a density of 1×10^7 in the freezing medium.
5. Transfer 1 mL of the cell suspension to sterile cryovials.
6. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the temperature should decrease at a rate of 1°C per minute.
7. Store in liquid nitrogen.

Check the viability and recovery of frozen cells 24 hours after storing vials in liquid nitrogen by following the procedure on page 11 for initiating culture from frozen stock.

Maintain and optimize cell cultures

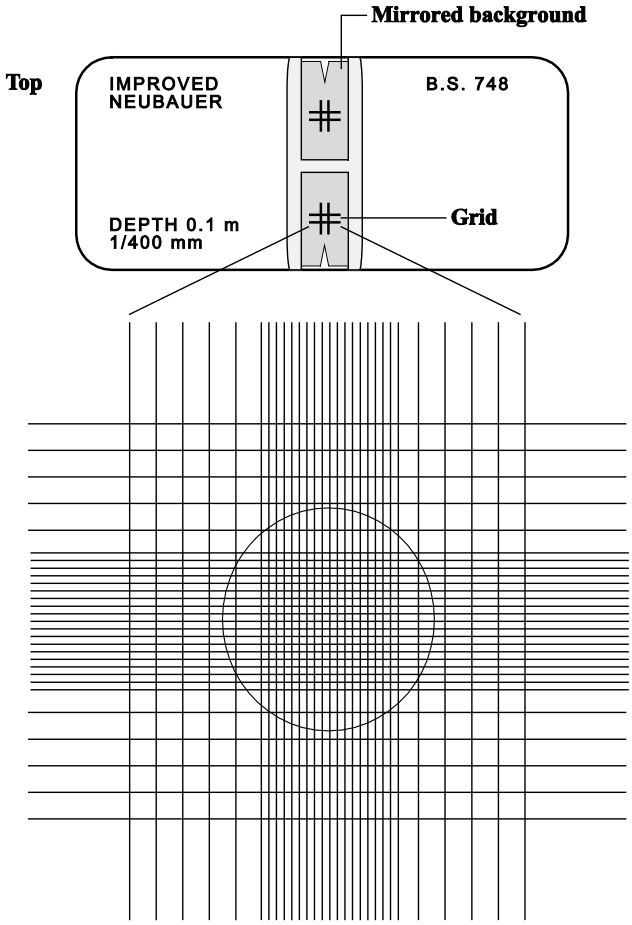
Introduction

Guidelines to maintain and optimize Mimic™ cell cultures are provided in this section. Included are protocols for cleaning spinner flasks.

Determine cell density—hemacytometer

Hemacytometers may be obtained from most major laboratory suppliers (e.g., Baxter Scientific). The procedure below provides some general directions on how to use the hemacytometer.

1. Clean the chamber and coverslip with alcohol. Dry and fix the coverslip in position.
2. Harvest cells. Add 10 µL of the cells to the hemacytometer. Do not overfill.
3. Place chamber in the inverted microscope under the 10X objective. Use phase contrast to distinguish cells.
4. Count the cells in the large, central gridded square (1 mm^2). The gridded square is circled in the graphic below. Multiply by 10^4 to estimate the number of cells per mL. Prepare duplicate samples and average the count.



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Determine cell viability—Trypan blue e xclusion

Trypan blue dye molecules are excluded by the membranes of viable cells, but readily enter dead cells. After staining with trypan blue, cells that are blue are considered nonviable.

The following procedure will enable you to quickly and accurately determine cell viability. Calculate viability by dividing the number of viable (non-blue) cells by the total number of cells in the grids on a hemacytometer.

1. Determine the cell density of your cell suspension using a hemacytometer (see previous page).
2. Prepare a 0.4% solution of trypan blue in buffered isotonic salt solution, pH 7.2 to 7.3 (i.e., phosphate-buffered saline).
3. Add 0.1 mL of trypan blue stock to 1 mL of cells.
4. Load a hemacytometer and examine immediately under a microscope at low magnification.
5. Count the number of blue staining cells and the number of total cells. Cell viability should be at least 95% for healthy log-phase cultures.

$$\%\ \text{viable cells} = \left[1.00 - \left(\frac{\text{Number of blue cells}}{\text{Number of total cells}}\right)\right] \times 100$$

To calculate the number of viable cells per mL of culture, use the formula below. Remember to correct for the dilution factor.

Number of viable cells $\times 10^4 \times 1.1$ = cells/mL culture

Clean spinner flasks

Proper spinner care is important for both cell health and to prevent cross-contamination of your cultures with different viral stocks. Baculovirus particles can survive a cycle through the autoclave. We have developed a protocol that uses two cycles in the autoclave to eliminate virus contamination of future spinner cultures.

1. Wash with 7X glass cleaner (Bellco), or substitute 10% acetic acid if necessary. Wash while spinning, approximately 2 hours. The 7X glass cleaner is preferable, as trace amounts are not detrimental to the cells. Acetic acid can be detrimental if not rinsed away completely.
   IMPORTANT: Do not use detergents**.**
2. Rinse 5 times with tap water.
3. Rinse 5 times with deionized water.
4. Tighten spinner cap and o-ring around impeller bar; spinner arm caps should be finger-tightened.
5. Autoclave once wet with deionized water for 45 minutes on liquid cycle.

   Note: You can repeat this step more than once if desired.
6. Autoclave once dry for 45 minutes on dry cycle.

Cell log

Cell line: Cell Lot #/Date of Freeze down:

Date of initiation: Medium: Medium Lot No.:

Passage No.	Date of Passage	Cell Density	Cell Viability	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				

Record any changes in medium and medium lot number in the "Comment" column as well as any notes about cell appearance.

Troubleshooting

Introduction

The following section summarizes the most common problems associated with insect cell culture. Morphological changes in the cells or changes in the growth rate can indicate an underlying problem with the culture. If you have kept a record of cell viabilities and doubling times, this can be helpful in diagnosing a problem when it comes up. Use this table as a general guide for potential problems-—what might cause them and what to do to solve and prevent them.

Adherent cells: Morphology changes—First week in culture

Observation	Possible cause	Recommended action
Cells are granular and/or floating	Mimic™ cells may become loosely attached or float, even when viable.	Transfer cells (including floaters) to suspension culture after required density has been attained (1×10^6 cells/mL). Check viability of floaters using trypan blue exclusion method (page 18).
	Medium was not removed within 1 hour of thawing cells. DMSO in the freezing medium can be harmful to cells.	Remove medium, add fresh medium.
	Cells are exhibiting serum sensitivity.	Try a new type or lot of serum, thaw new cells.
Cell lysis or debris	Aging cell culture with passage number greater than 30.	Thaw new cells of lower passage number.

Continued on next page

Adherent cells: Morphology changes—More than one week in culture

Observation	Possible cause	Recommended action
Decreased viability and growth rate	Contamination with wild-type or recombinant baculovirus. You will see lysis, debris and/or swelling of cells. Inclusions in the nucleus indicates wild-type infection.	**Discard the old culture and initiate a fresh culture.** Thaw new cells. Decontaminate hood and equipment. Make up new medium.
	Too much mechanical manipulation during subculturing.	Thaw new cells. Switch to another method of subculturing (e.g., sloughing).
Floaters (greater than 5–10% of cells in culture)	Mimic™ cells may become loosely attached or float, even when viable.	Transfer cells (including floaters) to suspension culture after required density has been attained (1×10^6 cells/mL). Check viability of floaters.
	Passaging too often before confluency.	Remove medium/floaters. Replace with fresh medium. Passage only at confluency.
	Cells are overgrown.	If this is the first time this has occurred, replace medium and split cells. Thaw new cells if this has happened more than once.
	Sensitivity to a new lot or brand of serum (FBS).	Test your culture with another serum. We recommend using serum from Gibco.
Cells swollen, spots in the nucleus	Contamination with wild-type or recombinant baculovirus.	**Discard the old culture and initiate a fresh culture.** Thaw new cells. Make new medium. Decontaminate equipment.

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Adherent cells: Growth and/or viability decrease

Observation	Possible cause	Recommended action
Cell doubling time is too long (>30 hours)	Cells have grown past confluency more than once.	Thaw new cells and check your cell cultures daily to avoid overgrowth.
	Cells are at a high passage number (greater than 30 passages).	Thaw new cells and make sure that you are freezing down cells at a low passage number (less than 10) so that new cultures are optimal.
	Cells are repeatedly being passaged before confluency.	Let cells grow to confluency for next passage. If doubling time does not increase, thaw new cells.
	Cells are split back below 20% confluency repeatedly.	Concentrate cells to a confluency of 50% or greater and plate out (see page 10).
Cell viability is less than 90%	Subculturing technique too harsh.	Thaw new cells and try using a different subculturing technique (e.g., sloughing, page 13).
	Bacterial or fungal contamination.	**Discard the old culture and initiate a fresh culture.** Decontaminate equipment. Prepare fresh medium.
	Contamination with wild-type or recombinant baculovirus. You will see lysis, debris and/or swelling of cells. Inclusions in the nucleus indicates wild-type infection.	**Discard the old culture and initiate a fresh culture.** Decontaminate equipment. Prepare fresh medium. Keep separate bottles of medium for cell culture and for virus work. Do not work with cell culture at the same time that you are working with virus in the tissue culture hood.

Continued on next page

Suspension Culture: Morphology Changes

Observation	Possible cause	Recommended action
Clumping of cells	Shake/spin rate is too slow.	Use 80–95 rpm.
Cell lysis or debris	Aging cell culture has been maintained in suspension longer than two months.	Thaw new cells of low passage number and initiate a new suspension culture.
	Cell shearing due to shaking/spinning.	Add Pluronic™ F-68 at 0.1% final concentration.
	Contamination with wild-type or recombinant baculovirus. You will see lysis, debris and/or swelling of cells. Inclusions in the nucleus indicates wild-type infection.	**Discard the old culture and initiate a fresh culture.** Thaw new cells and start a new culture. Decontaminate hood and equipment. Make up new medium. Keep separate bottles of medium for cell culture and viral work.
Cells swollen, spots in the nucleus	Contamination with wild-type or recombinant baculovirus.	See above recommendations for baculovirus contamination.

Suspension Culture: Growth and/or Viability Decrease

Observation	Possible cause	Recommended action
Cell doubling time is too long (>30 hours)	Culture seeded or split back to a cell density that is too low (less than 5×10^5 cell/mL).	Concentrate cells to 1×10^6 cell/mL (see page 10). This will boost them into log phase growth.
	Spinner overgrown to a cell density >3×10^6 cells/mL.	1. Split cells to a density of 1.5×10^6 cells/mL. 2. Grow them overnight to a density of 2.0×10^6 to 2.5×10^6 cells/mL. 3. Split them back to a density of 1.0×10^6 cells/mL, continue normal maintenance (see page 15).

Continued on next page

Spinner Culture: Growth and/or Viability Decrease

Observation	Possible cause	Recommended action
Cell doubling time is too long (>30 hours), continued	Inadequate aeration.	The volume of culture should not exceed 40% the designated capacity of the shake flask, or 50% the capacity of the spinner.
		Add Pluronic™ F-68 at 0.1% final concentration.
		Volume of culture should meet minimum volume requirements (i.e., 1×10^6 cells/mL).
		For spinner cultures, impeller should spin with a smooth continuous motion. Jerky or jumping motion of the impeller does not provide good aeration.
Viability < 90%	Cell shearing due to shaking/spinning.	Shaker/spinner speed is too high; recommended speed is 80–95 rpm. Add Pluronic™ F-68 at 0.1% final concentration.
	Inadequate aeration.	See solutions above for **Inadequate Aeration**.
	Bacterial or fungal Contamination.	**Discard the old culture and initiate a fresh culture.** Decontaminate hood and equipment. Make up new medium. Keep separate bottles of medium for cell culture and viral work. Try adding antibiotics to your medium.
	Contamination with wild-type or recombinant baculovirus. You will see lysis, debris and/or swelling of cells. Inclusions in the nucleus indicates wild-type infection.	**Discard the old culture and initiate a fresh culture.** Adequate sterilization of spinner flasks between uses where there is contact with baculovirus is required. See page 18.

Accessory products

Insect culture media

Mimic™ cells must be grown in Grace's Insect Medium, Supplemented, with serum. See below for ordering information:

Product	Amount	Catalog No.
Grace's Insect Medium, Supplemented	500 mL	11605-094
FBS, Heat-Inactivated	500 mL	10082-147
Grace's Insect Medium, Unsupplemented	500 mL	11595-030

Insect expression systems

Mimic™ cells can be used with a variety of insect expression systems; however, they are not compatible with Bac-N-Blue™ Baculovirus Expression System (see note on page 6).

Product	Amount	Catalog No.
Bac-to-Bac™ Expression System	1 kit	10359-016
InsectSelect™ Kit	1 kit	K800-01
InsectSelect™ BSD Kit	1 kit	K820-01

Transfection reagents

For optimal results, we recommend using Cellfectin™ II Reagent for transfection.

Product	Amount	Catalog No.
Cellfectin™ II Reagent	1 mL	10362-100

Additional products

The products listed below may be used with Mimic™ cells. For more information, refer to **thermofisher.com** or contact Technical Support (page 26).

Product	Amount	Catalog No.
Countess™ Automated Cell Counter (includes 50 Countess™ cell counting chamber slides and 2 mL of Trypan Blue Stain)	1 unit	C10227
LIVE/DEAD™ Cell Vitality Assay Kit	1000 assays	L34951
Trypan Blue Stain	100 ml	15250-061

Documentation and support

Customer and technical support

Visit thermofisher.com/support for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the Documentation and support section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at: **www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf**
- World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: **www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf**