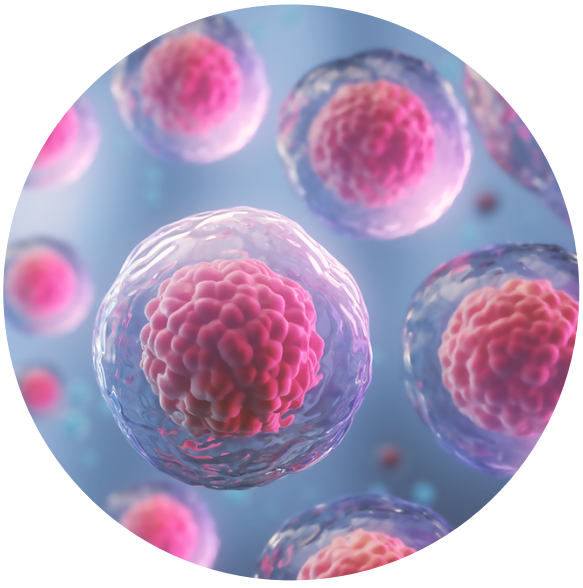
Instruction Manual

Human Female Reproductive Cell Systems

Human Uterine Smooth Muscle Cells (HUtSMC)
Human Uterine Fibroblasts (HUtF)
Human Endometrial (Uterine) Epithelial Cells (HEuEC)
Human Cervical Epithelial Cells (HCxEC)
Human Fallopian Tube Epithelial Cells (HFTEC)
Human Vaginal Epithelial Cells (HVEC)

This product is for Research Use Only. This product is not approved for human or veterinary use or for use in in vitro diagnostics or clinical procedures.



LIFELINE® CELL TECHNOLOGY | Better Solutions for Breakthrough Results

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LIFELINE® CELL TECHNOLOGY

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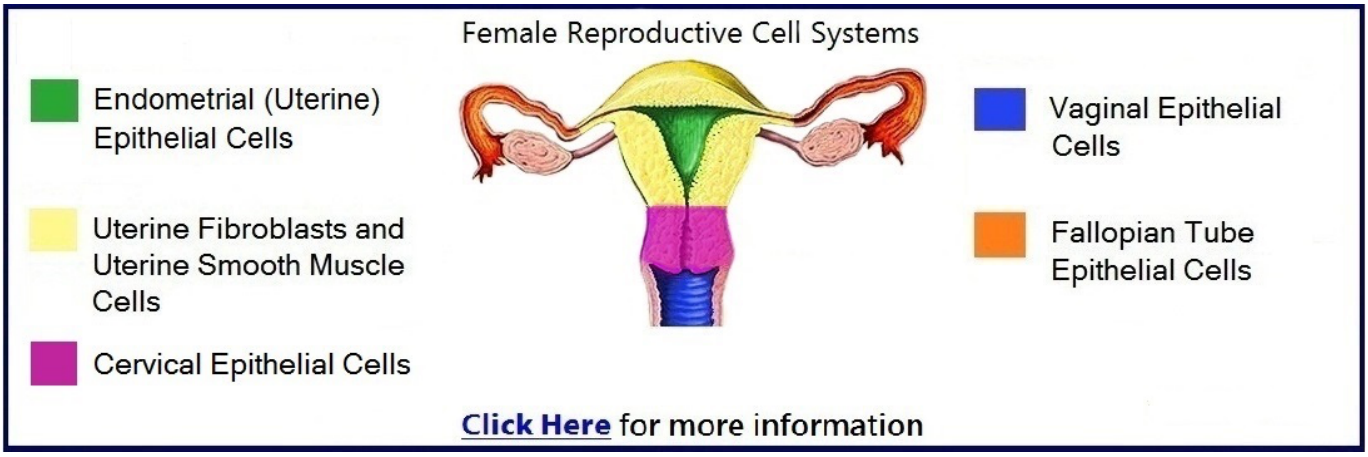

General Information

Shipping
Cells, and LifeFactors™ Kits are shipped on dry ice. Basal media are shipped at ambient temperature.

Safety and Use Statement
Lifeline® products are For Research Use Only. Lifeline products are not approved for human or veterinary use, for use in *in vitro* diagnostics, or clinical procedures.

Lifeline recommends storing cryopreserved vials in liquid nitrogen vapor phase, NOT in liquid phase. Handle cryopreserved vials with caution. Always wear eye protection and gloves when working with cell cultures. Aseptically vent any liquid nitrogen from cryopreserved vials by carefully loosening the vial cap in a biosafety cabinet prior to thawing the vials in a water bath. If vials must be stored in liquid phase, the vials should be transferred to liquid nitrogen vapor phase storage or in a -80°C freezer for up to 24 hours prior to being thawed.

Safe Handling of Cryopreserved Vials
Human Female Reproductive Cells are sold as cryopreserved vials and are shipped in insulated packages containing dry ice to ensure the cells remain in a cryopreserved state. To maintain the cells' integrity, unpack the products immediately upon receipt and store at a temperature lower than -150°C or in the vapor phase of a liquid nitrogen dewar. If the cells are to be thawed and plated within 24 hours, they may be stored at -80°C. Do not store the vial for more than 24 hours at -80°C as the cells will slowly degrade at this temperature.

Basic Aseptic Technique
Cells, medium, and LifeFactors should only be used in an aseptic environment, such as a Class II biological safety cabinet with front access and filtered laminar airflow, or an equivalent device. Always wear gloves and eye protection when working with these cell culture materials. Wipe or spray all bottles and vials with 70% ethanol or

isopropanol, especially around the area of the cap, before placing them in the biological safety cabinet. Allow these surfaces to dry completely before opening the bottle or vials. Transfer cells, medium, or LifeFactors with disposable sterile pipettes. Do not mouth pipette! Take up the volume needed into the pipette, being careful not to touch the sterile tip to the rim of the container or any other surface. Close the container and open the container into which the transfer is being made, again being careful not to touch any

surfaces with the sterile tip. Transfer the material and close the container. Wash your hands before and after working with cell cultures. Do not block airflow in a laminar flow hood as this may compromise sterility. Ensure that biological safety cabinets are certified routinely and that the HEPA filters are replaced regularly.

Lifeline Technical Note

There are different and often contradictory terminologies used by cell culture companies to define the passage number of cells. Lifeline's designation of 'primary cells' are cells that have been isolated from tissue, and expanded once in culture vessel(s) before being cryopreserved. Lifeline's designation of 'secondary cells' are cells that have been isolated from tissue, and expanded twice in culture vessel(s) before being cryopreserved. Lifeline's designation of 'tertiary cells' are cells that have been isolated from tissue, and expanded three times in culture vessel(s) before being cryopreserved. Lifeline's cells are cryopreserved at the earliest possible passage to ensure the highest viability, purity, and plating efficiency.

All donated tissues have been obtained under proper informed consent and adhere to the Declaration of Helsinki, The Human Tissue Act (UK), CFR Title 21, and HIPAA Regulations related to obtaining and handling human tissue for Research Use.

Quality Testing for Guaranteed Consistency and Reproducible Results

Lifeline Cell Technology manufactures products using the highest quality raw materials and incorporates extensive quality assurance in every production run. Exacting standards and production procedures ensure consistent performance.

The Lifeline Guarantee

Lifeline's rigorous quality control ensures sterility and performance to standardized testing criteria. Upon request, Lifeline will provide lot specific QC test results, material safety data sheets, and certificates of analysis. See complete guarantee/warranty statement at lifelinecelltech.com or contact your Lifeline representative for more information.

Product Ordering Information

Female Reproductive Cells

Part Number	Cell Type		Quantity of Cells per Vial Guaranteed
FC-0075	HUtSMC	Normal Human Uterine Smooth Muscle Cells	500,000
FC-0076	HUtF	Normal Human Uterine Fibroblasts	500,000
FC-0078	HEuEC	Normal Human Endometrial (Uterine) Epithelial Cells	500,000
FC-0080	HCxEC	Normal Human Cervical Epithelial Cells	500,000
FC-0081	HFTEC	Normal Human Fallopian Tube Epithelial Cells	500,000
FC-0083	HVEC	Normal Human Vaginal Epithelial Cells	500,000

Expansion Media

Part Number	Components	For Use With Cell Part Number(s)
LL-0001	FibroLife™ Serum-Free Medium Complete Kit (FibroLife Basal Medium, FibroLife Serum-Free LifeFactors™ Kit)	FC-0076
LL-0011	FibroLife S2 Medium Complete Kit (FibroLife Basal Medium, FibroLife S2 LifeFactors Kit)	FC-0076
LL-0014	VascuLife® SMC Complete Kit (VascuLife Basal Medium, VascuLife SMC LifeFactors Kit)	FC-0075
LL-0068	ReproLife™ Medium Complete Kit (ReproLife Basal Medium, ReproLife LifeFactors Kit)	FC-0078 FC-0083
LL-0072	ReproLife CX Medium Complete Kit (ReproLife CX Basal Medium, ReproLife CX LifeFactors Kit)	FC-0080 FC-0081

Additional Products

Part Number	Components
LS-1009	Phenol Red Supplement [33 mM], 1 mL
LS-1104	GA Antimicrobial Supplement, 0.5 mL (Gentamicin 30 mg/mL, Amphotericin B 15 µg/mL); *provided with purchase of LL-0001, LL-0011, LL-0014, LL-0068, or LL-0072*
LL-0013	TrypKit™ Subculture Reagent Kit (Phosphate Buffered Saline, 0.05% Trypsin/0.02% EDTA, Trypsin Neutralizing Solution)
LM-0015	FrostaLife™ Cryopreservation Solution, 100 mL

Female Reproductive Cell Culture Information

Cell Culture Characteristics

Part Number	Cell Type	Cryopreservation Passage	Normal Morphology	Population Doublings Guaranteed	Recommended Expansion Medium
FC-0075	HUtSMC	Secondary	Elongated, spindle-shaped, sheet-like	15	LL-0014
FC-0076	HUtF	Secondary	Bipolar or multipolar, elongated, spindle-shaped	15	LL-0001 LL-0011
FC-0078	HEuEC	Tertiary	Polygonal, cuboidal, monolayer	5	LL-0068
FC-0080	HCxEC	Tertiary	Polygonal, cuboidal, monolayer	10	LL-0072
FC-0081	HFTEC	Tertiary	Mixed cuboidal and elongated, clonal	5	LL-0072
FC-0083	HVEC	Tertiary	Polygonal, cuboidal, monolayer	10	LL-0068

Part Number	Cell Type	Recommended Seeding Density (cells/cm^2)	Recommended Passage at Percent Confluence (%)	Recommended Passaging (Split Ratio)	Approximate Number of Days per Passage
FC-0075	HUtSMC	2,500 to 5,000	80 to 90	1:4 to 1:12	5 to 9
FC-0076	HUtF	2,500 to 5,000	80 to 90	1:5 to 1:20	2 to 7
FC-0078	HEuEC	5,000	85 to 100	1:3 to 1:6	5 to 8
FC-0080	HCxEC	5,000 to 10,000	85 to 100	1:3 to 1:6	5 to 7
FC-0081	HFTEC	5,000 to 10,000	85 to 100	1:3 to 1:6	5 to 7
FC-0083	HVEC	5,000	85 to 100	1:3 to 1:6	3 to 7

Common Cell Culture Vessels

Culture Vessel Type*	Approximate Culture Area (cm^2) per Flask, Dish, or Well**	Approximate Volume of Medium (mL) per Flask, Dish, or Well***
T-12.5 flask	12.5	2.5 to 4
T-25 flask	25	5 to 8
T-75 flask	75	15 to 24
35 mm dish	8	1.6 to 2.6
60 mm dish	21	4.2 to 6.7
100 mm dish	55	11 to 17.6

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150 mm dish	148	29.6 to 47.4
6 well plate	9.5	1.9 to 3.1
12 well plate	3.8	0.8 to 1.2
24 well plate	1.9	0.4 to 0.6
48 well plate	0.95	0.2 to 0.3
96 well plate (flat bottom)	0.32	0.06 to 0.1

*The chart above demonstrates typical cell culture vessels. The chart is not inclusive of all possible cell culture vessels.

**The culture surface area values listed above are approximate and will vary depending upon the brand of culture vessels used. Please refer to manufacturers' specifications for actual values.

***Lifeline® recommends feeding cells with a minimum of 1 mL of the respective complete medium per 5 cm^2 culture surface area. Volume of medium and frequency of feeding should increase in proportion to the cell confluence.

Thawing and Expanding Female Reproductive Cells

Materials and Equipment Needed

1. Female Reproductive Cell cryovial(s)
2. Respective Basal Medium and LifeFactors™ Kit (see table of Expansion Media on page 2 for details)
3. Supplies for counting viable cells (e.g. Trypan Blue, hemacytometer)
4. Tissue culture treated vessel(s)
5. 70% ethanol or isopropanol for disinfecting surfaces
6. 37°C water bath
7. Cell culture incubator (37°C, 5% CO_2, humidified)
8. Biosafety cabinet

Preparing Culture Medium (see individual media instructions for more detail)

1. Thaw frozen supplements at room temperature or in a 37°C water bath.
2. Spray the bottle(s) and vial(s) with 70% ethanol or isopropanol and transfer them to a biosafety cabinet.
3. Aseptically add the labeled volume of each LifeFactor to the respective bottle of basal medium using a pipette.
4. A vial of Gentamicin and Amphotericin B (GA; LS-1104) is provided with the purchase of LL-0001, LL-0011, LL-0014, LL-0068, or LL-0072 for your convenience. The use of GA is recommended to inhibit potential fungal or bacterial contamination of eukaryotic cell cultures.
5. Cap the bottle securely and mix supplemented medium by gently swirling or inverting bottle.
6. Do not shake or froth the medium.
7. The supplemented medium may be stored at 2 to 8°C for up to two weeks.

Pre-warming Medium

1. If using less than 100 mL of complete medium, Lifeline® recommends warming only the volume needed in a sterile conical tube. Repeated warming of the entire bottle over extended periods will cause degradation of the medium and reduced shelf life.
2. Medium will warm to 37°C in 10 to 20 minutes, depending on the volume. Do not leave medium in water bath for extended periods.

Thawing and Plating Cryopreserved Cells

1. Remove the vial from storage in the vapor phase of a liquid nitrogen dewar and check the cap to be sure that the vial is securely sealed.
2. Spray the vial with 70% ethanol or isopropanol and transfer it to a biosafety cabinet.
3. Allow it to dry thoroughly and carefully loosen the cap a quarter turn to vent any liquid nitrogen that may have entered the vial.
4. Recap the vial and hold only the bottom half of the vial in a 37°C water bath for approximately 1 minute, or until the vial is almost completely thawed—a small amount of ice should still be visible.
5. Dry the thawed vial, spray the exterior of the vial with 70% ethanol or isopropanol and place the vial in a biological safety cabinet and allow it to dry.

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6. Gently resuspend the cells in the vial using a 1 or 2 mL sterile pipette. Do not centrifuge; the cells may be directly plated from the vial.
7. Using a clean hemacytometer, perform a cell count. Lifeline recommends using Trypan Blue during the cell count to calculate the viability of the cells post thaw.
8. Plate the cells into pre-warmed culture medium in the desired culture vessel(s) at the recommended cell density (see the table on page 3 for details).
9. Gently rock the culture vessel(s) from side to side and front to back to evenly distribute cells within the vessel(s).
10. Place seeded culture vessel(s) in a 37°C, 5% CO_2, humidified incubator.
11. Replace the culture medium after the cells have attached, approximately 4 to 36 hours after inoculation to remove cryopreservation reagents.
12. Change the culture medium per the recommended feeding guidelines specific to the cell type. Lifeline recommends that the volume of culture medium and frequency of medium changes increase in proportion to the cell confluence. Please see pages 7 through 12 for the table of Recommended Feeding Guidelines respective to the cell type being fed.

Passaging/Subculturing Cells

Materials and Equipment Needed

1. Female Reproductive Cell culture(s)
2. Respective Basal Medium and LifeFactors™ Kit (see table of Expansion Media on page 2 for details)
3. TrypKit™ Subculture Reagent Kit (LL-0013)
    a. Phosphate Buffered Saline, without Ca^{2+}, without Mg^{2+} (PBS; CM-0001)
    b. 0.05% Trypsin/0.02% EDTA (CM-0017)
    c. Trypsin Neutralizing Solution (TNS; CM-0018)
4. Sterile, disposable conical-bottom centrifuge tube(s)
5. Supplies for counting viable cells (e.g. Trypan Blue, hemacytometer)
6. Tissue culture treated vessel(s)
7. 70% ethanol or isopropanol for disinfecting surfaces
8. 37°C water bath
9. Cell culture incubator (37°C, 5% CO_2, humidified)
10. Biosafety cabinet

Passaging Cells

1. All steps must be completed under aseptic conditions in a biological safety cabinet.
2. Female Reproductive Cells may be passaged when the culture reaches the recommended confluence (as listed in the table on page 3) and actively proliferating.
3. Lifeline® recommends using TrypKit Subculture Reagent Kit (LL-0013).
4. Aspirate the medium from the culture vessel(s).
5. Rinse the vessel(s) with PBS (CM-0001) by adding at least 1 mL of PBS per 5 cm^2.
6. Aspirate the PBS from the culture vessel(s), repeat the rinse if desired.
7. Trypsinize the cells with Lifeline's 0.05% Trypsin/0.02% EDTA (CM-0017) by adding at least 2 mL per 25 cm^2 to the vessel(s). Make sure all cells are coated with the Trypsin/EDTA.
8. Observe the cells carefully under the microscope. When the cells contract, they are ready to be released. This normally takes at least 2 to 3 minutes depending on the confluence of the cells and the temperature. Do not trypsinize longer than needed to detach the cells as this may damage the cells.
9. Once the cells are fully detached, add Lifeline's Trypsin Neutralizing Solution (TNS; CM-0018) using a volume equal to the amount of Trypsin/EDTA that was originally used.
10. Using aseptic laboratory techniques pipette the cells into a sterile centrifuge tube.
11. Collect the remaining cells by rinsing the culture vessel(s) with PBS (1 mL per 5 cm^2) and pipetting the cells into the sterile centrifuge tube.
12. Check culture vessel(s) under the microscope for cells still attached and repeat steps if necessary to retrieve all the cells from the vessel(s).
13. Centrifuge cells at 150 x g^* for 3 to 5 minutes. For best results, calculate speed for individual centrifuge type.
    Do not centrifuge cells longer or at higher speeds than necessary as this will cause cell damage.
14. After centrifugation, the cells should form a clean loose pellet.

15. Aspirate neutralized trypsin from the centrifuge tube and gently resuspend the cell pellet in the respective pre-warmed culture medium.
16. Using a clean hemacytometer, perform a cell count. Lifeline recommends using Trypan Blue during the cell count to calculate the viability of the cells.
17. Inoculate new culture vessel(s) at the recommended cell density (see the table on page 3 for details).
18. Gently rock the culture vessel(s) from side to side and front to back to evenly distribute cells within the vessel(s).
19. Place newly seeded culture vessel(s) in a 37°C, 5% CO_2, humidified incubator.
20. Replace the culture medium approximately 24 to 36 hours after inoculation.
21. Change the culture medium per the recommended feeding guidelines specific to the cell type. Lifeline recommends that the volume of culture medium and frequency of medium changes increase in proportion to the cell confluence. Please see pages 7 through 12 for the table of Recommended Feeding Guidelines respective to the cell type being fed.
22. Please consult Lifeline's technical service department if issues arise from trypsinization or centrifugation.

***To calculate Relative Centrifugal Force (x *g*)**

$$\mathbf{RCF = 0.00001118 \times (rpm)^2 \times r}$$

r = rotational radius in centimeters
rpm = rotations or revolutions per minute

Cryopreserving Cells

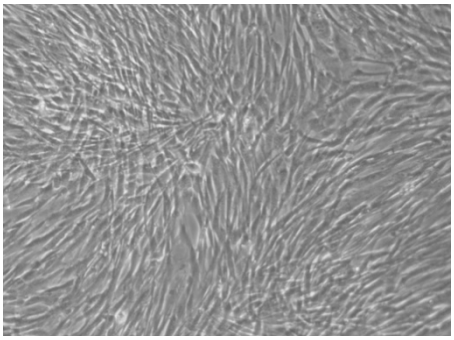
Materials and Equipment Needed

1. Female Reproductive Cell culture(s)
2. Respective Basal Medium and LifeFactors™ Kit (see table of Expansion Media on page 2 for details)
3. TrypKit™ Subculture Reagent Kit (LL-0013)
    a. Phosphate Buffered Saline, without Ca^{2+}, without Mg^{2+} (PBS; CM-0001)
    b. 0.05% Trypsin/0.02% EDTA (CM-0017)
    c. Trypsin Neutralizing Solution (TNS; CM-0018)
4. Sterile, disposable conical-bottom centrifuge tube(s)
5. Supplies for counting viable cells (e.g. Trypan Blue, hemacytometer)
6. FrostaLife™ Cryopreservation Solution (LM-0015)
7. Or to make your own cryopreservation medium, combine:
    a. 80% (v/v) fully supplemented expansion medium (see table of Expansion Media on page 2 for details)
    b. 10% (v/v) Fetal Bovine Serum (FBS), sterile
    c. 10% (v/v) Dimethyl sulfoxide (DMSO), sterile
8. Sterile cryovials(s), appropriately labeled for traceability
9. 70% ethanol or isopropanol for disinfecting surfaces
10. Isopropanol freezing chamber
11. Ultra-Low freezer (-70°C to -80°C)
12. Biosafety cabinet

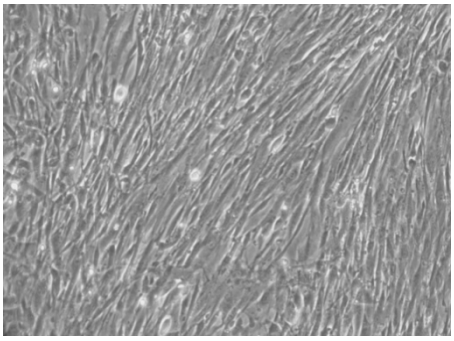
Cryopreservation of Cells

1. Detach cells following the Passaging/Subculturing procedure beginning on the previous page.
2. After centrifugation, resuspend the cells in a minimal volume of the respective expansion medium (e.g. approximately 100 µL per 25 cm^2).
3. Using a clean hemacytometer, perform a cell count. Lifeline® recommends using Trypan Blue during the cell count to calculate the viability of the cells prior to cryopreservation.
4. Dilute the cells to a final cell concentration of 0.5 x 10^6 cells/mL using FrostaLife Cryopreservation Solution or other cryopreservation medium.
5. Immediately dispense the cells into sterile cryovial(s) and freeze using a controlled rate freezer or an isopropanol chamber at -80°C overnight.
6. Transfer the cryovial(s) to the vapor phase of a liquid nitrogen storage dewar at ≤ -130°C.

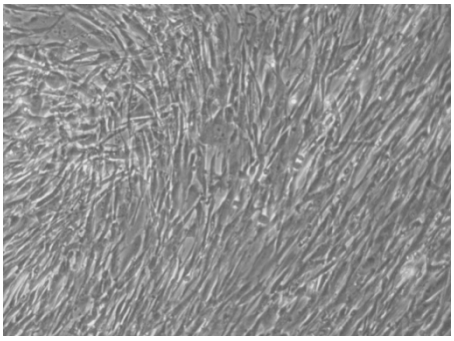
Normal Human Uterine Smooth Muscle Cells



HUtSMC, passage 3, 6 days after inoculation with 2,500 cells/cm^2 (100X).



HUtSMC, passage 4, 8 days after inoculation with 2,500 cells/cm^2 (100X).



HUtSMC, passage 5, 8 days after inoculation with 2,500 cells/cm^2 (100X).

Cell Culture Characteristics

Part Number	Cell Type	Cryopreservation Passage	Normal Morphology	Population Doublings Guaranteed	Recommended Expansion Medium
FC-0075	HUtSMC	Secondary	Elongated, spindle-shaped, sheet-like	15	LL-0014

Part Number	Cell Type	Recommended Seeding Density (cells/cm^2)	Recommended Passage at Percent Confluence (%)	Recommended Passaging (Split Ratio)	Approximate Number of Days per Passage
FC-0075	HUtSMC	2,500 to 5,000	80 to 90	1:4 to 1:12	5 to 9

Expansion Medium

Part Number	Components	For Use With Cell Part Number(s)
LL-0014	VascuLife® SMC Complete Kit (VascuLife Basal Medium, VascuLife SMC LifeFactors™ Kit)	FC-0075

Recommended Feeding Guidelines

Guidelines for a T-25 Flask. Adjust volumes according to culture surface area.
Every other day, remove medium and feed with 5 mL of fresh supplemented medium.
Most cultures which are 50% confluent will be ready for passage within two days and should be fed with 7 to 8 mL of supplemented medium.
Do not use more than 10 mL of medium per 25 cm^2 of culture surface to ensure that the media depth is at a level where gas diffusion will be sufficient to support the cells' requirements for oxygen.

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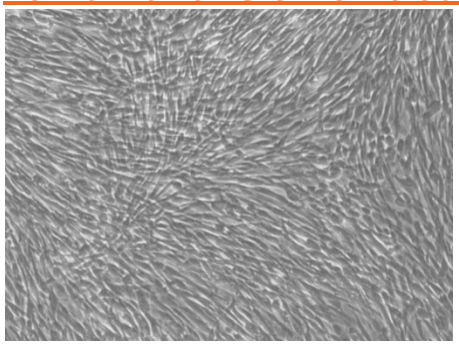
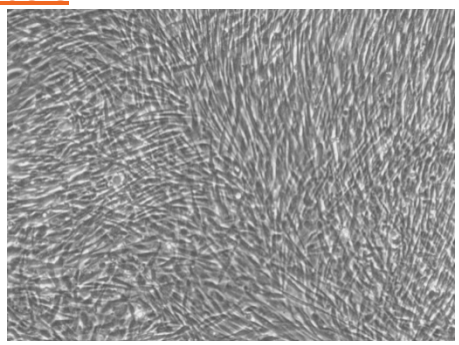
The depth of the medium affects gas diffusion gradients through the culture medium to the cells. The volumes of medium recommended in this table result in a range of depths between 2 mm and 5 mm, which is compatible with general recommendations, 10 mL being at the maximum depth allowable (5 mm).

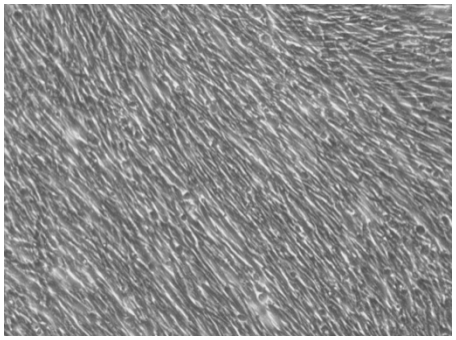
Normal Human Uterine Fibroblasts



HUtF, passage 3, 2 days after inoculation with 2,500 cells/cm^2 (100X).



HUtF, passage 4, 6 days after inoculation with 2,500 cells/cm^2 (100X).



HUtF, passage 5, 6 days after inoculation with 2,500 cells/cm^2 (100X).

Cell Culture Characteristics

Part Number	Cell Type	Cryopreservation Passage	Normal Morphology	Population Doublings Guaranteed	Recommended Expansion Medium
FC-0076	HUtF	Secondary	Bipolar or multipolar, elongated, spindle-shaped	15	LL-0001 LL-0011

Part Number	Cell Type	Recommended Seeding Density (cells/cm²)	Recommended Passage at Percent Confluence (%)	Recommended Passaging (Split Ratio)	Approximate Number of Days per Passage
FC-0076	HUtF	2,500 to 5,000	80 to 90	1:5 to 1:20	2 to 7

Expansion Media

Part Number	Components	For Use With Cell Part Number(s)
LL-0001	FibroLife™ Serum-Free Medium Complete Kit (FibroLife Basal Medium, FibroLife Serum-Free LifeFactors™ Kit)	FC-0076
LL-0011	FibroLife S2 Medium Complete Kit (FibroLife Basal Medium, FibroLife S2 LifeFactors Kit)	FC-0076

Recommended Feeding Guidelines

Guidelines for a T-25 Flask. Adjust volumes according to culture surface area.
Every other day, remove medium and feed with 5 mL of fresh supplemented medium.

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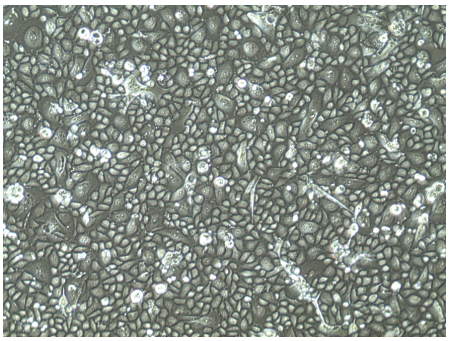
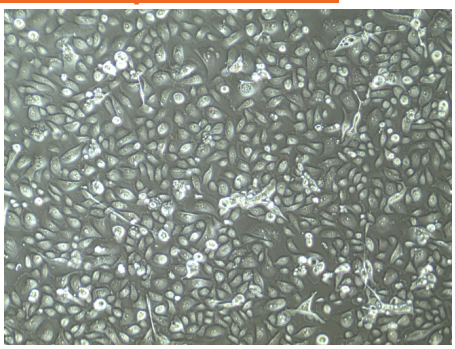
Most cultures which are 50% confluent will be ready for passage the following day and should be fed with 7 to 8 mL of supplemented medium.
Do not use more than 10 mL of medium per 25 cm^2 of culture surface to ensure that the media depth is at a level where gas diffusion will be sufficient to support the cells' requirements for oxygen.

The depth of the medium affects gas diffusion gradients through the culture medium to the cells. The volumes of medium recommended in this table result in a range of depths between 2 mm and 5 mm, which is compatible with general recommendations, 10 mL being at the maximum depth allowable (5 mm).

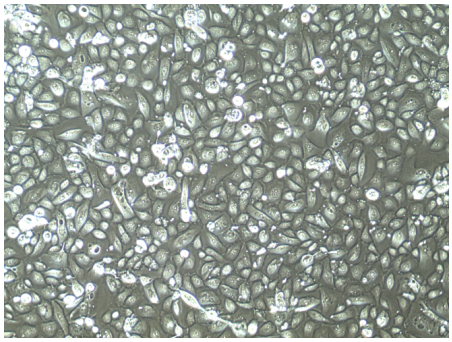
Normal Human Endometrial (Uterine) Epithelial Cells



HEuEC, passage 4, 7 days after inoculation with 6,150 cells/cm^2 (100X).



HEuEC, passage 5, 5 days after inoculation with 10,000 cells/cm^2 (100X).



HEuEC, passage 6, 7 days after inoculation with 10,000 cells/cm^2 (100X).

Cell Culture Characteristics

Part Number	Cell Type	Cryopreservation Passage	Normal Morphology	Population Doublings Guaranteed	Recommended Expansion Medium
FC-0078	HEuEC	Tertiary	Polygonal, cuboidal, monolayer	5	LL-0068

Part Number	Cell Type	Recommended Seeding Density (cells/cm^2)	Recommended Passage at Percent Confluence (%)	Recommended Passaging (Split Ratio)	Approximate Number of Days per Passage
FC-0078	HEuEC	5,000	85 to 100	1:3 to 1:6	5 to 8

Expansion Medium

Part Number	Components	For Use With Cell Part Number(s)
LL-0068	ReproLife™ Medium Complete Kit (ReproLife Basal Medium, ReproLife LifeFactors™ Kit)	FC-0078

Recommended Feeding Guidelines

Guidelines for a T-25 Flask. Adjust volumes according to culture surface area.
Every other day, remove medium and feed with 5 mL of supplemented medium. **However, when cultures reach 50% (or greater) confluence, remove medium and feed with 5 to 8 mL of supplemented medium daily.**

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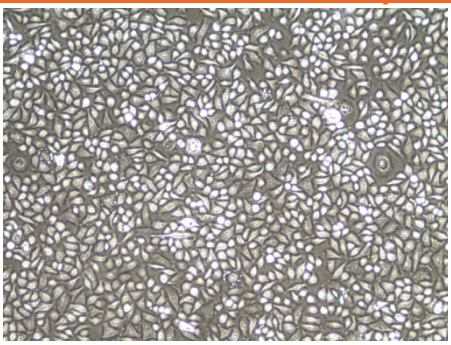
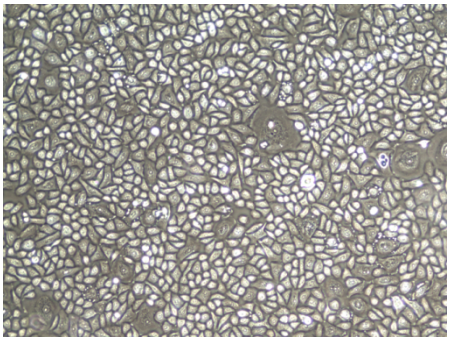
Most cultures which are 50% confluent will be ready for passage in the following 1 to 2 days and should be fed with 7 to 8 mL of supplemented medium daily.
Do not use more than 10 mL of medium per 25 cm^2 of culture surface to ensure that the media depth is at a level where gas diffusion will be sufficient to support the cells' requirements for oxygen.

The depth of the medium affects gas diffusion gradients through the culture medium to the cells. The volumes of medium recommended in this table result in a range of depths between 2 mm and 5 mm, which is compatible with general recommendations, 10 mL being at the maximum depth allowable (5 mm).

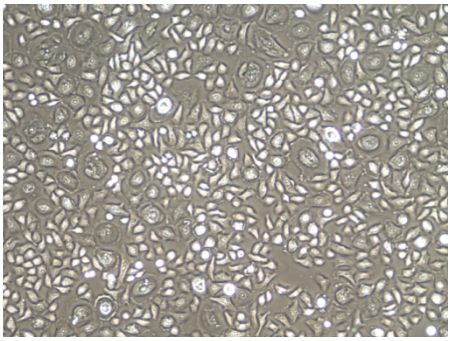
Normal Human Cervical Epithelial Cells



HCxEC, passage 4, 5 days after inoculation with 5,000 cells/cm^2 (100X).



HCxEC, passage 5, 5 days after inoculation with 5,000 cells/cm^2 (100X).



HCxEC, passage 6, 5 days after inoculation with 5,000 cells/cm^2 (100X).

Cell Culture Characteristics

Part Number	Cell Type	Cryopreservation Passage	Normal Morphology	Population Doublings Guaranteed	Recommended Expansion Medium
FC-0080	HCxEC	Tertiary	Polygonal, cuboidal, monolayer	10	LL-0072

Part Number	Cell Type	Recommended Seeding Density (cells/cm^2)	Recommended Passage at Percent Confluence (%)	Recommended Passaging (Split Ratio)	Approximate Number of Days per Passage
FC-0080	HCxEC	5,000 to 10,000	85 to 100	1:3 to 1:6	5 to 7

Expansion Medium

Part Number	Components	For Use With Cell Part Number(s)
LL-0072	ReproLife™ CX Medium Complete Kit (ReproLife CX Basal Medium, ReproLife CX LifeFactors™ Kit)	FC-0080

Recommended Feeding Guidelines

Guidelines for a T-25 Flask. Adjust volumes according to culture surface area.
Every other day, remove medium and feed with 5 mL of supplemented medium. **However, when cultures reach 50% (or greater) confluence, remove medium and feed with 5 to 8 mL of supplemented medium daily.**

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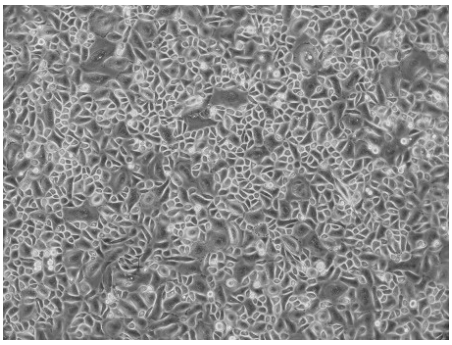
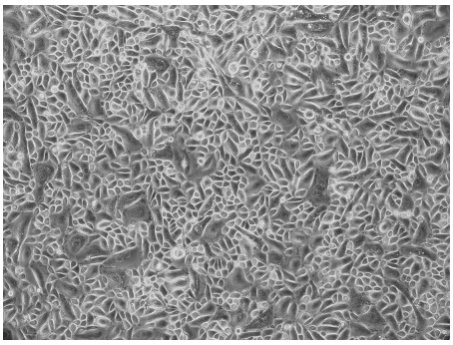
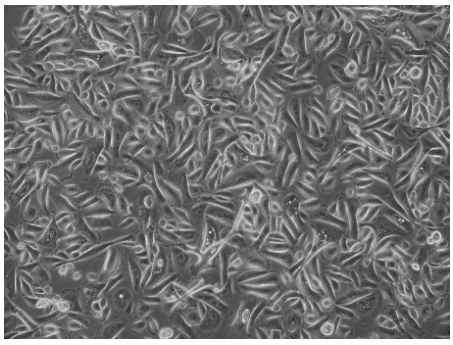


HFTEC, passage 4, 5 days after inoculation with 5,000 cells/cm² (100X).

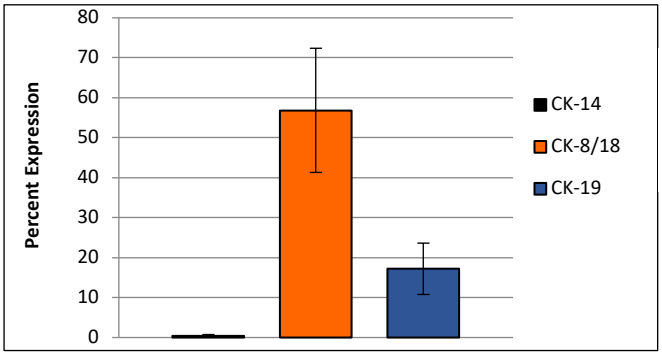


HFTEC, passage 5, 6 days after inoculation with 5,000 cells/cm² (100X).



HFTEC, passage 6, 7 days after inoculation with 5,000 cells/cm² (100X).

Normal Human Fallopian Tube Epithelial Cells



Average Cytokeratin Expression for HFTEC.

HFTEC have been characterized by FACS as dominantly positive for Cytokeratin-8/18.

Cell Culture Characteristics

Part Number	Cell Type	Cryopreservation Passage	Normal Morphology	Population Doublings Guaranteed	Recommended Expansion Medium
FC-0081	HFTEC	Tertiary	Mixed cuboidal and elongated, monolayer	5	LL-0072

Part Number	Cell Type	Recommended Seeding Density (cells/cm²)	Recommended Passage at Percent Confluence (%)	Recommended Passaging (Split Ratio)	Approximate Number of Days per Passage
FC-0081	HFTEC	5,000 to 10,000	85 to 100	1:3 to 1:6	7 to 14

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Expansion Medium

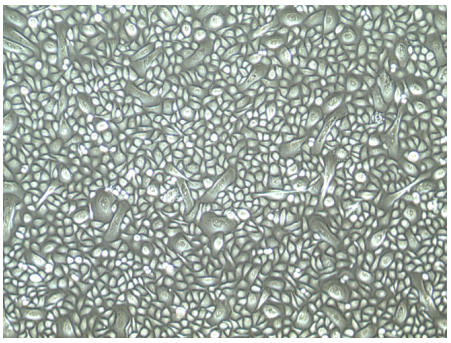
Part Number	Components	For Use With Cell Part Number(s)
LL-0072	ReproLife™ CX Medium Complete Kit (ReproLife CX Basal Medium, ReproLife LifeFactors™ Kit)	FC-0081

Recommended Feeding Guidelines

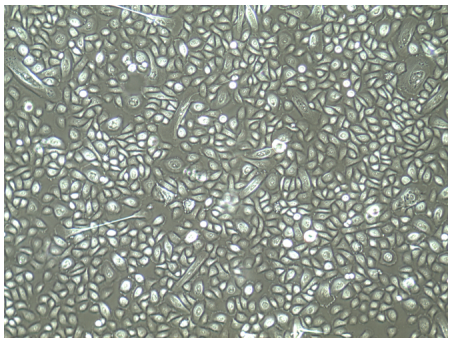
Guidelines for a T-25 Flask. Adjust volumes according to culture surface area.
Every other day, remove medium and feed with 5 mL of supplemented medium. **However, when cultures reach 50% (or greater) confluence, remove medium and feed with 5 to 8 mL of supplemented medium daily.**
Most cultures which are 50% confluent will be ready for passage in the following 2 to 5 days and should be fed with 7 to 8 mL of supplemented medium daily.
Do not use more than 10 mL of medium per 25 cm^2 of culture surface to ensure that the media depth is at a level where gas diffusion will be sufficient to support the cells' requirements for oxygen.

The depth of the medium affects gas diffusion gradients through the culture medium to the cells. The volumes of medium recommended in this table result in a range of depths between 2 mm and 5 mm, which is compatible with general recommendations, 10 mL being at the maximum depth allowable (5 mm).

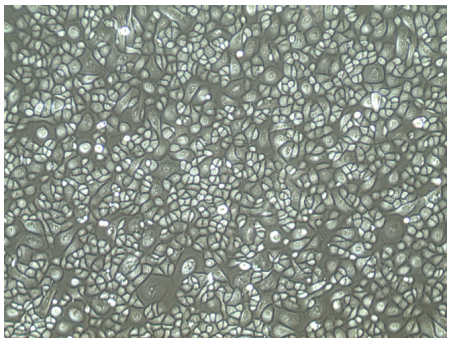
Normal Human Vaginal Epithelial Cells



HVEC, passage 4, 5 days after inoculation with 7,950 cells/cm^2 (100X).



HVEC, passage 5, 3 days after inoculation with 10,000 cells/cm^2 (100X).



HVEC, passage 6, 4 days after inoculation with 10,000 cells/cm^2 (100X).

Cell Culture Characteristics

Part Number	Cell Type	Cryopreservation Passage	Normal Morphology	Population Doublings Guaranteed	Recommended Expansion Medium
FC-0083	HVEC	Tertiary	Polygonal, cuboidal, monolayer	10	LL-0068

Part Number	Cell Type	Recommended Seeding Density (cells/cm²)	Recommended Passage at Percent Confluence (%)	Recommended Passaging (Split Ratio)	Approximate Number of Days per Passage
FC-0083	HVEC	5,000	85 to 100	1:3 to 1:6	3 to 7

Expansion Medium

Part Number	Components	For Use With Cell Part Number(s)
LL-0068	ReproLife™ Medium Complete Kit (ReproLife Basal Medium, ReproLife LifeFactors™ Kit)	FC-0083

Recommended Feeding Guidelines

Guidelines for a T-25 Flask. Adjust volumes according to culture surface area.
Every other day, remove medium and feed with 5 mL of supplemented medium. **However, when cultures reach 50% (or greater) confluence, remove medium and feed with 5 to 8 mL of supplemented medium daily.**
Most cultures which are 50% confluent will be ready for passage in the following 1 to 2 days and should be fed with 7 to 8 mL of supplemented medium daily.
Do not use more than 10 mL of medium per 25 cm² of culture surface to ensure that the media depth is at a level where gas diffusion will be sufficient to support the cells' requirements for oxygen.

The depth of the medium affects gas diffusion gradients through the culture medium to the cells. The volumes of medium recommended in this table result in a range of depths between 2 mm and 5 mm, which is compatible with general recommendations, 10 mL being at the maximum depth allowable (5 mm).

Troubleshooting

Problem	Likely cause	Solution
Few or no viable cells when thawing a vial of Female Reproductive Cells	Original cryovial was not stored correctly	• Order a new vial of Lifeline's Female Reproductive Cells and store in vapor phase liquid nitrogen until ready to use. • Keep cells in a -80°C freezer or on dry ice the day of use and when ready to thaw immediately transfer them to a 37°C water bath.
	"Home-made" cryopreserved Female Reproductive Cells	• Cryopreserve cells between 0.5 x 10^6 to 1 x 10^6 cells/mL. • Cryopreservation volume less than 1 mL per vial can negatively impact viability upon subsequent thaw.
		• Cryopreserve cells at low passage.
		• Follow procedures for cryopreservation exactly. • See page 6 for procedural details.
		• Obtain a new vial of Female Reproductive Cells.
Poor cell attachment	Incorrect thawing medium	• Use pre-warmed, fully supplemented medium that is correct for the type of Female Reproductive Cell being cultured. • See page 2 for media recommendations.
	Cells were not inoculated at the correct density	• Lifeline® recommends inoculating each of the Female Reproductive Cell types at a specific seeding density. • See page 3 for seeding density recommendations.
Cells grow slowly	Incorrect growth medium	• Use pre-warmed, fully supplemented medium that is correct for the type of Female Reproductive Cell being cultured. • See page 2 for media recommendations.
	Seeding density for Female Reproductive Epithelial Cells was too low	• Lifeline recommends using a seeding density range of 5,000 to 10,000 cells/cm^2. • Please refer to the table of Common Cell Culture Vessels provided on page 3 for assistance in seeding density calculations.
	Cells are too old	• Lifeline recommends using HUtSMC, or HUtF within 3 or 4 passages post receipt. Do not allow the cells to become over confluent. • Lifeline recommends using HEuEC, or HFTEC within 1 to 2 passages post receipt. Please note these cells have a finite limit of peak proliferation. Do not allow the cells to become over confluent. • Lifeline recommends using HCxEC, or HVEC within 2 to 3 passages post receipt. Please note these cells have a finite limit of peak proliferation. Do not allow the cells to become over confluent.

Female Reproductive Epithelial Cells appear to have “blebs”	Cells are hungry	• Volume of medium and frequency of feeding should increase in proportion to the cell confluence. • For cultures lower than 50% confluence, Lifeline recommends feeding cells with a minimum of 1 mL of the respective complete medium per 5 cm^2 culture surface area, every other day. • For cultures greater than 50% confluence, Lifeline recommends feeding cells with a minimum of 1.5 to 2 mL of the respective complete medium per 5 cm^2 culture surface area, every day.

For any question on medium supplementation or cell feeding guidelines; please contact technical service at 877.845.7787. We are here to help.

Notes: