CellSystems®
Biotechnologie Vertrieb GmbH

LIFELINE CELL TECHNOLOGY

Normal Human Mitomycin C Treated Fibroblasts (MCFibs®) Instruction Sheet

Safety and Use Statement

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 recommends storing cryopreserved vials in liquid nitrogen vapor phase. Handle cryopreserved vials with caution. Always wear eye protection and gloves when working with cell cultures. Aseptically vent any 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 vapor phase storage or -80°C for up to 24 hours prior to being thawed.

Basic Aseptic Technique

Medium and LifeFactors® should only be used in an aseptic environment, 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 materials. Wipe or spray medium bottle and LifeFactors with 70% ethanol or isopropanol before opening, especially around the area of the cap. Allow these surfaces to dry completely before opening the bottle or vials. Transfer of medium or LifeFactors should be done 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.

Cryopreserved Vials

MCFibs® 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 liquid nitrogen vapor phase. 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.

Medium Preparation

FibroLife® Basal Medium contains no growth factors, antimicrobials or phenol red. To support cell viability and function you must add FibroLife Serum-Free LifeFactors (LS-1010) or FibroLife S2 LifeFactors (LS-1038) to the basal medium (see FibroLife Medium instructions). Antimicrobials and phenol red are not required for cell proliferation, but may be purchased separately.

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Pre-warming Medium

If using less than 100 mL of medium, Lifeline® recommends warming only the volume needed in a sterile conical tube. Repeated warming of the entire bottle over extended periods may cause degradation of the medium and reduced shelf life. When warming the entire bottle of medium, Lifeline recommends using a Lifeline water bath sleeve (included with medium) to help protect the medium from contaminants in the 37°C water bath. Medium will warm to 37°C in 10-20 minutes, depending on the volume. Do not leave medium in water bath for extended periods.

Preparing Gelatin Coated Vessels

Culture vessels should be prepared with an attachment factor such as gelatin prior to thawing the MCFibs®. To prepare the gelatin coated culture vessels add 1.0 mL of 0.1% gelatin per each 10 cm^2 of culture surface area using standard sterile protocol. Rock culture vessel to coat the entire culture surface of the vessel and incubate at 37°C without CO_2 for at least 30 minutes. An incubator with 5% CO_2 may be used in place of an incubator without CO_2. Gelatin coated vessels may be left in the incubator overnight. Before MCFibs are to be inoculated, aspirate excess gelatin from the culture vessel using sterile procedures. Add 1.0 mL of FibroLife complete medium per 5 cm^2 of culture vessel surface area. Gelatin coated vessels should then be placed in a 37°C incubator with or without 5% CO_2 for at least one hour to equilibrate prior to inoculating MCFibs.

Thawing and Plating Cryopreserved Cells

Remove vial from dewar and check the cap to be sure that the vial is securely sealed. Spray the vial with 70% ethanol or isopropanol and transfer it to a biosafety cabinet. Allow it to dry thoroughly and carefully loosen the cap to vent any liquid nitrogen that may have entered the vial. Recap the vial and hold only the bottom half of the vial in a 37°C water bath for approximately one minute or until vial is almost completely thawed—a small amount of ice should still be visible. To avoid potential contamination, do not allow the vial cap to make contact with the water. Do not over thaw as this may damage the cells. Dry the 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. Carefully remove the cap to avoid contamination or spatter. 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. Plate the cells into pre-warmed fully supplemented FibroLife Serum-Free Medium or FibroLife S2 Medium in the desired culture vessel at a density of 20,000 to 40,000 cells per cm^2. (See Standard Calculation for Plating of Cells on the next page.) Flasks with vented caps, commonly referred to as filter caps, are strongly recommended. Gently rock the culture vessel from side to side and front to back to evenly distribute cells within the vessel. Place seeded culture vessel in a 37°C, 5% CO_2 incubator. Refeed the cells after they have attached. Refeeding should occur 4-36 hours after inoculation to remove cryopreservation reagents.

Establishing a Feeder Layer With Mitomycin C Treated Fibroblasts

Thaw and re-suspend cryopreserved MCFibs according to thawing instructions. Remove gelatin coated culture vessels containing fresh pre-warmed FibroLife medium and place in a biological safety cabinet just prior to thawing and re-suspending cells. Inoculate MCFibs suspension directly into the gelatin-coated flask, swirl gently to ensure even distribution. Recommended MCFibs density for the feeder layer is 20,000 to 40,000 cells per cm^2 of surface area. Culture vessel should remain undisturbed in the incubator until the MCFibs have attached and flattened. This will take a minimum of 4 hours, however, Lifeline recommends incubating overnight for best results. Some debris and dead cells are common once the healthy cells have attached. If McFibs feeder layer are not to be used within 36 hours of inoculation aspirate the medium from the culture vessel and replace with pre-warmed FibroLife Medium every 24 hours. MCFibs feeder layers are best when used within one to three days after plating.

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Inoculation of MCFibs® Feeder Layer With Proliferating Cells

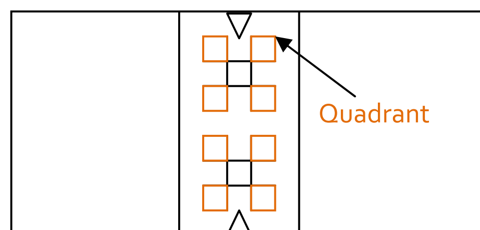
Before adding proliferating cells to the MCFibs feeder layer, first aspirate the medium from the culture vessel. Add pre-warmed medium appropriate for the proliferating cell type to be cultured. Return the culture vessel to the incubator and allow at least 30 minutes for the system to equilibrate and recover. The feeder layer is now ready to be inoculated with the proliferating cells. Follow standard procedures for the proliferating cell type from this point forward.

Standard Calculation for Plating of Cells

Gently re-suspend the cells evenly in the medium. Using a clean hemacytometer and sterile technique, remove 20 µL of the cell suspension to a separate tube, such as a microcentrifuge tube. Add an equal volume of 0.4% Trypan Blue solution to the cell suspension in the microcentrifuge tube and allow it to sit for 1 to 5 minutes. Place 10 µL of the cell suspension into one chamber of the hemacytometer. Count a minimum of 4 quadrants on the hemacytometer (see diagram below). Dead and dying cells are permeable to Trypan Blue, viable cells will not be blue. For accurate cell counts, optimal number of cells per quadrant should be 25-75 cells. After counting the cells, calculate the average of the 4 quadrants. Take the cell count average and multiply by any dilution factor and by 10^4 to get the number of cells per mL. Multiply the desired seeding density (20,000-40,000) viable cells per cm^2) by the surface area of the vessel(s) to be inoculated. This will give you the total number of cells to inoculate one vessel. Divide the number of cells needed to inoculate one vessel by the total number of cells in the cell suspension. This will give you the volume of cell suspension with which to inoculate each vessel. Inoculate the cells into the culture vessels prepared with pre-warmed culture medium. Mix gently to evenly distribute the cells and place culture vessels into the incubator at 37°C, 5% CO_2.

Sample calculation:
Average viable cells per quadrant = 31
31 cells/quadrant x 10,000 quadrants/mL x 2 (dilution factor*) = 620,000 cells/mL
*if using equal volumes of Trypan Blue and cell suspension.

Inoculating a T-75 flask at 2500 cells/cm^2:
2500 x 75 = 187,500 cells/flask
Calculate volume of cell suspension to inoculate each flask with:
187,500 cells/flask divided by 620,000 cells/mL = 0.302 mL/flask



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Quick Steps for Feeder Layer Set Up:

1. Always wash hands before and after working with cell cultures.
2. Always wear eye protection and gloves when working with cell cultures.
3. Store cryopreserved cells in liquid nitrogen vapor phase upon receipt.
4. When working with cells or medium, always use a certified biological safety cabinet.
5. Handle cryopreserved vials with caution. Aseptically vent any nitrogen from cryopreserved vials in a biosafety cabinet prior to thawing in a water bath.
6. Prepare 0.1% gelatin solution for coating culture vessel.
7. Coat vessel with gelatin using 1.0 mL of gelatin solution per 10 cm^2.
8. Incubate at 37°C with 0% CO_2 (5% may be used) for a minimum of 30 minutes prior to use. Incubate overnight for best results.
9. Aspirate 0.1% gelatin solution from vessel and add 1.0 mL of FibroLife® Complete Medium for each 5cm^2.
10. Incubate for 30 minutes to one hour at 37°C with 5% CO_2 to equilibrate.
11. Thaw MCFibs, and inoculate gelatin-coated culture vessels containing pre-warmed FibroLife complete medium at a density of 20,000 to 40,000 cells per cm^2.
12. Incubate at 37°C with 5% CO_2 until cells have attached and flattened, four hours minimum, overnight is preferred.
13. Aspirate medium and debris from prepared feeder layer.
14. Add pre-warmed medium specific to the proliferating cells to be cultured on the feeder layer, return to incubator for a minimum of 30 minutes.
15. Inoculate vessel containing the feeder layer with proliferating cells at a density specific to the proliferating cell type.
16. For any question on cell handling, please contact CellSystems® technical service. We are here to help.

Catalog and Ordering Information:

Product	Catalog Number(s)
Cryopreserved Normal Human Mitomycin C Treated Fibroblasts (3,000,000 cells/vial)	FC-0002
FibroLife® Serum-Free Medium Complete Kit (FibroLife Basal Medium, FibroLife LifeFactors® Kit)	LL-0001
FibroLife® S2 Medium Complete Kit (FibroLife Basal Medium, FibroLife LifeFactors® Kit)	LL-0011

Notes:

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