

FibroLife® Xeno-Free^S Complete Cell Culture System Instruction Sheet



Safety and Use Statement

These products are for Research Use Only. These products are not approved for human or veterinary use or for use in *in vitro* diagnostics or clinical procedures.

Many of these products contain human serum and should be handled as potentially infectious material. While the human serum used in this product has been tested, no known test method can guarantee the absence of Hepatitis B virus, Hepatitis C virus, HIV-1, HIV-2 or other infectious agents. All products which contain or have contacted human blood based

products should be handled as Biosafety Level 2 (cdc.gov) and according to the Bloodborne Pathogen Standard 29 CFR 1910.1030.

Medium Storage

FibroLife Xeno-Free Complete Medium should be stored at -20°C until it is needed, and then stored at 2 to 8°C for up to one month. Users should take care to protect the medium from extended exposure to light. Do not use product beyond expiration date. Multiple freeze/thaw cycles are not recommended.

Medium Preparation

FibroLife Xeno-Free Complete Medium contains all the necessary growth factors to support fibroblast growth. However, FibroLife Xeno-Free Complete Medium contains no antimicrobials, and no phenol red. A vial of Xeno-Free Penicillin/Streptomycin (XF-PS) is included in FibroLife Xeno-Free Complete Culture Kit (LL-0048) for your convenience. Xeno-Free phenol red is not available at this time.

Product	Part No.	Volume	Storage
FibroLife Xeno-Free Complete Medium	LM-0013	500 mL	-20°C until ready to use. Thaw and store at 2-8°C for up to 1 month.
Components of FibroLife Xeno-Free Complete Medium			
Xeno-Free Growth Factors	Concentration	Xeno-Free Growth Factors	Concentration
L-Glutamine	7.5 mM	rh FGF basic	5 ng/mL
Hydrocortisone Hemisuccinate	1 µg/mL	rh EGF	5 ng/mL
Ascorbic Acid	50 µg/mL	rh TGF-β1	30 pg/mL
Insulin	5 µg/mL	Human Serum	2%

Xeno-Free Kits	Part No.	Unit	Storage
FibroLife Xeno-Free Complete Culture Kit	LL-0048	Kit	Store as per each item's label.
FibroLife Xeno-Free Complete Medium	LM-0013	500 mL	-20°C
Phosphate Buffered Saline (Xeno-Free)	CM-0001	500 mL	Room Temperature
Trypsin/EDTA Xeno-Free	CM-0046	100 mL	-20°C
Trypsin Neutralizing Solution Xeno-Free	CM-0047	100 mL	-20°C
FrostaLife™ Xeno-Free Cryopreservation Solution	LM-0019	100 mL	-20°C
Xeno-Free Antimicrobial Supplement: Penicillin/Streptomycin (XF-PS)	LS-1073	1 mL	-20°C Penicillin 10,000 Units/mL Streptomycin 10,000 µg/mL
Xeno-Free Fibroblast Starter Kit (Contains one of: LL-0048, and FC-0037)	LL-0049	Kit	Store as per each item's label.
TrypKit™ Xeno-Free (Contains one of: CM-0001, CM-0046, and CM-0047)	LL-0043	Kit	Store as per each item's label.

To place an order, please visit lifelinecelltech.com or call technical and customer service at 877.845.7787.

Cells Supported by FibroLife® Xeno-Free^s Complete Medium:

- Xeno-Free Human Dermal Fibroblasts, neonatal (FC-0037)

Basic Aseptic Technique

Medium, and reagents 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 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 medium or reagents 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.

Thawing Medium

Thaw the FibroLife Xeno-Free Complete Medium overnight at 2 to 8°C; 500 mL bottles may take two days to thaw. Alternatively, the medium may be placed in a 25 to 37°C water bath to thaw. 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.

Mix FibroLife Xeno-Free Complete Medium by gently inverting the unopened bottle. Do not shake or froth the medium. The presence of slight precipitation may be seen after thawing the frozen medium; however, it will not affect Lifeline's guaranteed cell performance. The thawed medium may be stored at 2 to 8°C for up to one month.

Pre-warming Medium

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 and reduced shelf life of the medium. Medium will warm to 37°C in 10 to 30 minutes, depending on the volume. Do not leave medium in water bath for extended periods of time. 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 a 37°C water bath.

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 that 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 supplemented medium per 25 cm² of culture surface to ensure that the media 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).

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Optional Supplements

Xeno-Free[§] Penicillin-Streptomycin (XF-PS):

XF-PS is used to minimize bacterial contamination. These antimicrobials react with cells and may inhibit optimal growth. If proper aseptic technique is used, antimicrobials should not be necessary.

If you wish to add XF-PS to FibroLife[®] Xeno-Free Complete Medium, addition of 1 mL of XF-PS to 100 mL of media will provide an effective concentration to inhibit bacterial cell division.

A 1 mL supplement of XF-PS (LS-1073) is included in the FibroLife Xeno-Free Complete Culture Kit (LL-0048) for your convenience. XF-PS may also be purchased separately. XF-PS is best stored at -20°C; it should only be thawed once and stored at 4°C for a maximum of two weeks after thaw.

The Lifeline[®] Guarantee

Lifeline's rigorous quality control ensures sterility and performance to standardized testing criteria. Upon request, we 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 technical representative for more information.

[§]Lifeline documents that all materials used in the manufacture of products which are labeled 'Xeno-Free' have never come into contact with material of animal (non-human) origin and are not of animal (non-human) origin. FibroLife Xeno-Free Complete Medium contains human serum.

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

Notes:

Call Lifeline Technical Service and Sales at [877.845.7787](tel:877.845.7787)
or visit lifelinecelltech.com for more information

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