HiFibroXL™ Fibroblast Expansion Medium, Reduced Serum

Product Code: AL525

Product description:

HiFibroXL™ Fibroblast Expansion Medium is a reduced serum medium used for *in vitro* cultivation and expansion of Human Adult Dermal Fibroblasts (HADF) and Human Dermal Fibroblasts from Juvenile Foreskin. It contains basal medium (Part A) and fibroblast growth supplement (Part B). Part A consists of inorganic, organic salts, amino acids, vitamins and sodium bicarbonate. Part B consists of growth factors and nutrients necessary for growth of fibroblasts. This medium and supplement is devoid of antibiotics and antimycotics.

Products Required But Not Supplied

1. Media Supplements	Code
Antibiotic-Antimycotic Solution 100X [or] Gentamicin-Amphotericin B solution 1000X	A002 A031
2. Reagents for Sub-culture	Code
Dulbecco's Phosphate Buffered Saline (DPBS)	TL1006
Trypsin-EDTA Solution 1X	TCL128
Trypan Blue 0.5% Solution	TCL005
Soyabean Trypsin Inhibitor	TCL068

Directions:

1. Thaw fibroblast growth supplement (Part B) overnight at 2-8°C.
   Note: Precipitates in Part B after thawing are normal. Precipitates will not affect the performance of the medium.
2. Disinfect the external surface of the bottles of part A and Part B by spraying with isopropyl alcohol before placing in a bisafety hood.
3. Transfer the entire content of Part B to basal medium (Part A) under aseptic condition.
   Note: If desired, 5ml of antibiotic-antimycotic solution (A002) can be added to 500ml of complete medium.
4. Tightly cap the bottle and swirl gently to ensure proper mixing.
   Note: Do not mix vigorously. Doing so will cause formation of foam.
5. Store the complete medium at 2 - 8°C until use.

Quality control:

Appearance
Part A: Orangish red coloured clear solution
Part B: Pale yellow coloured clear solution

pH
7.00-7.60

Osmolality in mOsm/Kg H_2O
280.00-320.00

Sterility
No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

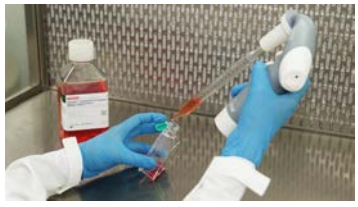
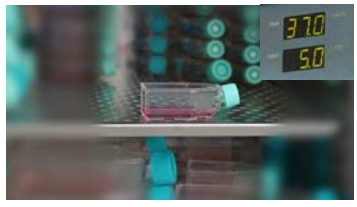
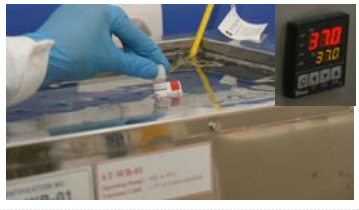
Cultural Response
The medium is tested for optimal cell growth and proliferation of fibroblast cells.

Storage and shelf life:

Store basal medium at 2-8°C away from bright light. Store endothelial progenitor growth supplement (Part B) at -20 °C. Use before expiry date given on the product label. Shelf life of the complete medium is 4 weeks at 2-8°C.
Note: Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.

Table 1 : Protocol for Thawing

- Cryopreserved cells are supplied in liquid nitrogen dry vapor shipper (-150°C to -130°C).
- Upon receipt, immediately transfer the vial to the vapor phase of liquid nitrogen tank.
- Store it in the tank until further use. Cells must be processed at least in a BSL II hood.

		Key Points to Remember	Time Required (approx.)
1. Preparation of Culture Vessel			
a. Add 5ml of complete medium to a T-25 flask		**Preparation of complete medium** **AL525 (Part A 500 ml) + (Part B 11.4 ml) + A002 (5 ml)**	60 secs
b. Place the flask at 37°C to equilibrate the medium			30 min
2. Thawing Procedure		**Make sure water bath is set at 37°C before starting the thawing procedure**	
a. Remove cryovial from the liquid nitrogen tank/ shipper wearing appropriate protective gear		**Thawing should be AS FAST AS POSSIBLE to minimize cell damage**	
b. Immediately thaw the vial partially by holding in a water bath at 37°C		**DO NOT hold the vial in water bath for more than 90-120 secs** **AVOID getting water upto the cap of the vial**	90-120 secs
c. Disinfect the vial by swabbing thoroughly with 70% isopropyl alcohol			10 secs
d. Add the cell suspension drop by drop to the T-25 flask containing the pre-warmed complete medium. e. Keep swirling the flask while adding the cell suspension		**DO NOT centrifuge cell suspension** **Dropwise addition is required to prevent the cells from stress induced by exothermic reaction**	30-60 secs

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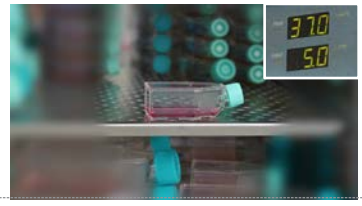
f. Cap the flask and shake gently to ensure proper mixing and uniform distribution of cells in the medium			10 secs
3. Incubation			
a. Incubate the cells at 37°C and 5% CO_2		**Check for cell attachment in 2-3 hrs**	2-3 hrs
b. If more than 70-80% cells are attached, replace the medium with fresh medium		**Medium change after 2-3 hours is mandatory to remove traces of DMSO** **If cells have not attached, centrifuge the cell suspension at 1000 rpm for 7-8 mins and resuspend in fresh medium**	60-120 secs 7-8 min
c. Incubate the cells at 37°C and 5% CO_2			3-5 days
YOUR CELLS ARE READY TO SUB-CULTURE			
4. Maintenance			
a. Monitor the cells every day b. Change the medium every alternate day c. Sub-culture once cells reach 70 - 80% confluence		**Use the recommended freezing medium for cryopreservation of cells.** **DO NOT allow the cells to reach 100% confluency before sub- culture or cryopreservation.** **In case of reduced serum or serum free media, use trypsin inhibitor solution (TCL068) for neutralization of Trypsin during subculture. Usage of just medium for neutralization will result in inefficient neutralization and will stress the cells resulting in reduced viability and cell death.**	

Table 2 : Sub-culture

- HADF /Human Dermal Fibroblasts from Juvenile Foreskin can be sub-cultured at a seeding density of 5000-10,000 cells/cm^2.
- Sub-culturing ratios can vary from 1:2 - 1:5
- A confluent T-25 flask of HADF /Human Dermal Fibroblasts from Juvenile Foreskin yields 1.0 x 10^6 cells

		Key Points to Remember	Time Required (approx.)
a. Aspirate entire medium and discard. DO NOT disturb the monolayer			60 secs
b. Wash the cells with 2-3 ml DPBS to remove residual medium. c. Aspirate off the DPBS and discard.		**Prior to use, make sure that Trypsin-EDTA solution is equilibrated to room temperature**	60 secs
d. Add 0.5 ml pre-warmed Trypsin-EDTA solution.		**Gently rock the flask to ensure complete coverage of the Trypsin-EDTA solution over the cells**	
e. Incubate the flask at 37°C for 30 - 60 secs.		**Exposing the cells to Trypsin-EDTA for longer time leads to loss of cell viability**	30-60 secs
f. Microscopically monitor the flask. g. When the cells start rounding up, gently tap the flask to ensure complete detachment of cells.			15 secs
h. To neutralize action of trypsin add 3 ml of complete medium, if 10% or more serum is supplemented to medium. i. Pipette gently to get a homogenous mixture of cells. j. If reduced serum medium AL525 is used, add 0.5 ml Soyabean Trypsin Inhibitor Solution (TCL068). k. Centrifuge the cell suspension at 1000 rpm for 10 mins. l. Discard supernatant and resuspend pellet in fresh 3 ml of complete medium by pipetting		**Vigorous pipetting will stress the cells**	60 secs

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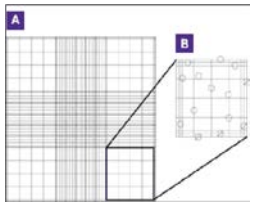
		Key Points to Remember	Time Required (approx.)
m. Count cells using hemocytometer n. Seed at recommended seeding density in a new flask containing fresh complete medium Refer to Table 3		**DO NOT refrigerate cells after splitting** **Seed immediately**	10-15 mins
o. Incubate in a humidified incubator at 37°C and 5% CO_2			48 hrs
Maintenance			
a. Monitor the cells every day			
b. Change the medium every alternate day			
c. Sub-culture once cells reach 70 - 80% confluence			

Table 3 : Seeding Density

Flask	Recommended Seeding Density	No. of Cells Per Flask	Volume of Medium (ml)
T-25	5000 cells/cm^2	0.125 x 10^6	5 - 7
	10,000 cells/cm^2	0.25 x 10^6	5 - 7

These are recommended seeding densities from literature and our studies. Higher seeding densities do not cause any harm to the cells and reduce the required population doublings per passage. Lower seeding densities may cause cells to lose viability, detach during culture and in general take more population doublings to reach confluence.

Related products:

Product name	Code with packing
HiFi™ Adult Dermal Fibroblasts (HADF)	CL005-0.5 CL005-T25 CL005-T75
HiFi™ Human Dermal Fibroblasts from Juvenile Foreskin	CL011-0.5 CL011-T25 CL011-T75
Accutase™	TCL075-1X100ML TCL075-5X100ML TCL075-1X500ML
Trypsin-EDTA Solution 1X	TCL033-5X100ML TCL033-2X500ML TCL033-6X500ML
Trypsin Inhibitor from soybean 1X; Liquid	TCL068-1X100ML TCL068-5X100ML
Dulbecco's Phosphate Buffered Saline	TL1006-5X100ML TL1006-2X500ML TL1006-6X500ML TL1006-18X500ML TL1006-1X1000ML
Antibiotic Antimycotic solution 100X, Liquid	A002-5X20ML A002-5X50ML A002-5X100ML
Gentamycin Solution	A005-5X20ML A005-5X50ML

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