HiMedia CellCulture
Enabling Breakthroughs

Product Information

HiEndoXL™ Endothelial Expansion Medium, Reduced Serum

Without BBE and VEGF

Product Code: AL530

Product description:

HiEndoXL™ Endothelial Expansion Medium is a reduced serum medium used for *in vitro* cultivation and expansion of Human Umbilical Vein Endothelial Cells (HUVEC). This medium enables superior growth of endothelial cells as compared to serum supplemented classical medium. It contains (Part A) and endothelial 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 endothelial cells. This medium and supplement is devoid of antibiotics, antimycotics, BBE and VEGF.

Products Required But Not Supplied

1. Media Supplements	
Antibiotic-Antimycotic Solution 100X [or]	A002
Gentamicin-Amphotericin B solution 1000X	A031
2. Reagents for Sub-culture	
Dulbecco's Phosphate Buffered Saline (DPBS)	TL1006
Trypsin/EDTA Solution 1X	TCL007
EnVzyme™ Easy	TCL137
Trypan Blue 0.5% solution	TCL005
Trypsin Inhibitor from Soyabean	TCL068
3. Reagent for Coating Culture vessel	
0.5% Gelatin solution in DPBS	TCL109

Directions:

1. Thaw endothelial cell growth supplement (Part B) overnight at 2-8ºC.
   Note: Few precipitates in Part B may be visible after thawing. 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 biosafety 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 - 8ºC until use. Complete medium can be directly used for culturing cells and no further addition of serum is required.

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 endothelial cells.

Storage and shelf life:

Store basal medium at 2-8°C away from bright light. Store endothelial cell growth supplement at -20°C. Use before expiry date given on the product label. Shelf life of the complete medium after reconstitution is 6 weeks at 2-8°C. Complete medium should be equilibrated at room temperature before adding to cells. **Note**: Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.

Table 1 : Gelatin Coating of Culture Vessel

		Key Points to Remember	Time Required (approx.)
		For uniform coating , make sure that the incubator is properly levelled	
Aseptically add 0.5% gelatin solution (TCL109)		**Refer Table 2 for recommended volumes of gelatin solution**	1 min
Incubate overnight at 37°C incubator			Overnight
Aspirate gelatin solution with the help of pipette.			
GELATIN COATED CULTURE VESSEL IS READY FOR USE			
If vessel is not used immediately, store at 2-8° upto one week		**Flask should be kept with caps tightly closed and plates should be sealed with a parafilm during storage**	

Table 2 : Recommended Volumes of Gelatin Solution for Different Culture Vessels

Culture Vessel	Volume Per Well
96-well plate	75 µl
48-well plate	150 µl
24-well plate	300 µl
12-well plate	500 µl
6-well plate	1 ml
T-25 Flask	5 ml
T-75 Flask	10 ml

Table 3 : Protocol for Thawing

- Cryopreserved cells are supplied in liquid nitrogen dry vapour 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.

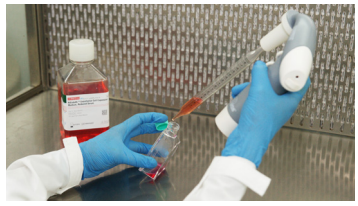
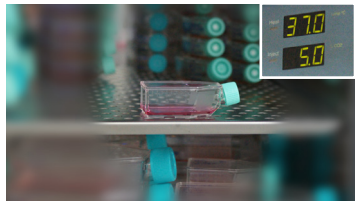
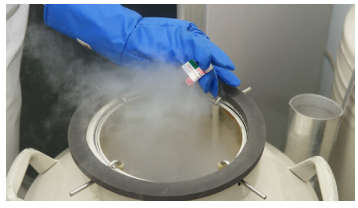
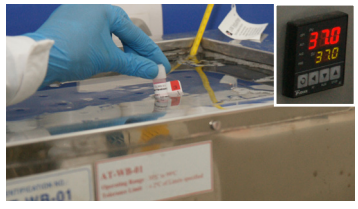
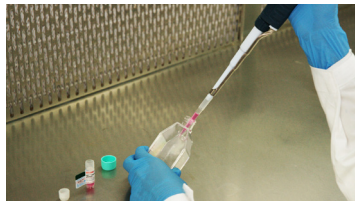
		Key Points to Remember	Time Required (approx.)
1. Preparation of Culture Vessel			
a. Add 5ml of complete medium to a T-25 flask		**Preparation of complete medium** AL530 (Part A 500 ml) + (Part B 20 ml) + A002 (5 ml)	60 secs
b. Place the flask at 37°C to equilibrate the medium			30 mins
2. Thawing Procedure		**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 up to 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. Keep swirling the flask while adding the cell suspension		**Dropwise addition is required to prevent the cells from stress induced by exothermic reaction**	30-60 secs

Table 3 : Protocol for Thawing

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- 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.

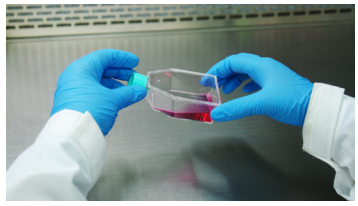
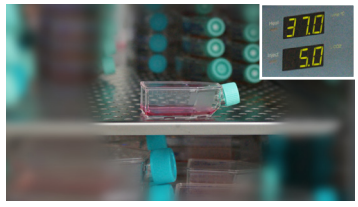
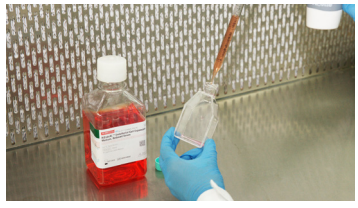
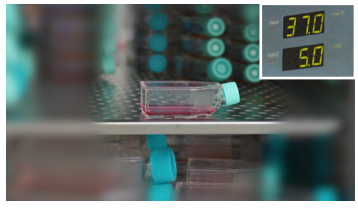
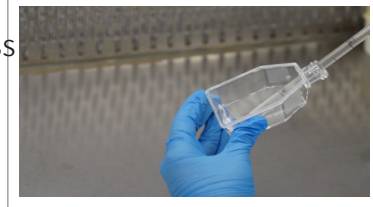
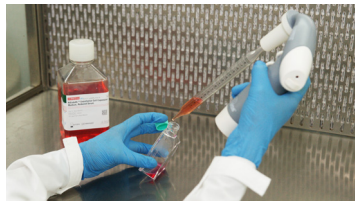
		Key Points to Remember	Time Required (approx.)
e. Cap the flask and shake gently to ensure proper mixing and uniform distribution of cells in the medium			10 secs
3. Incubation			
a. Incubate the cells at 37°C and 5% CO_2		**Check for cell attachment in 2-3 hrs**	2-3 hrs
b. If more than 70-80% cells 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 c. Sub-culture once cells reach 70 - 80% confluence		**Use the recommended freezing medium for cryopreservation of cells** **Upto 50% Confluency:** **Change the medium on alternate day** **After 50% Confluency:** **Change the medium everyday**	

Table 4 : Subculture

- HUVEC 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 HUVEC yields approx. 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 dissociation solution is equilibrated to room temperature**	60 secs
d. Add 0.5 ml pre-warmed Trypsin-EDTA solution or 1mL prewarmed EnVzyme™ Easy solution e. **Incubate the flask at 37°C** - Trypsin-EDTA solution dissociates HUVEC in approx. 30 sec - EnVzyme™ Easy solution dissociates HUVEC in approx. 7-10 min		**Gently rock the flask to ensure complete coverage of the dissociation solution over the cells** **Exposing the cells to Trypsin for longer time leads to loss of cell viability** **EnVzyme™ Easy is gentle on cells and longer exposure does not harm the cells. It does not require neutralization**	30 sec 7-10 mins
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. When using Trypsin-EDTA, neutralize its action by adding equal amount of Soyabean Trypsin Inhibitor Solution (TCL068). i. When using EnVzyme™ Easy, add 1mL complete medium j. Pipette gently to get a homogenous mixture of cells		**Vigorous pipetting will stress the cells**	60 secs

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- Sub-culturing ratios can vary from 1:2 - 1:5
- A confluent T-25 flask of HUVEC yields approx. 1.0 x 10^6 cells

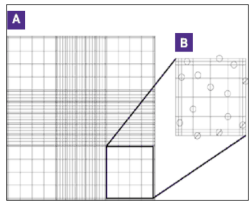
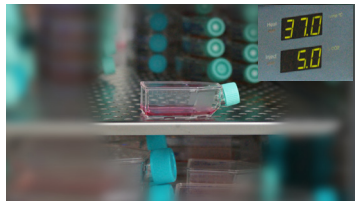
		Key Points to Remember	Time Required (approx.)
k. Count cells using hemocytometer l. Seed at recommended seeding density in a new flask containing fresh complete medium. Refer to Table 5		**DO NOT refrigerate cells after splitting** **Seed immediately**	10-15 mins
m. Incubate in a humidified incubator at 37°C and 5% CO_2			48 hrs
Maintenance			
a. Monitor the cells every day b. Change the medium c. Sub-culture once cells reach 70 - 80% confluence		**Upto 50% Confluency:** **Change the medium on alternate day** **After 50% Confluency:** **Change the medium everyday**	

Table 5 : 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	Packing
HiFi™ Human Umbilical Vein Endothelial Cells (HUVEC)	CL002-0.5 CL002-T25	0.5 million cells/vial 1 T25cm^2 flask
HiFi™ Human Umbilical Vein Endothelial Cells (HUVEC) Without BBE and VEGF	CL021-0.5 CL021-T25	0.5 million cells/vial 1 T25cm^2 flask
Accutase™	TCL075-1X100ML TCL075-5X100ML TCL075-1X500ML	1 x 100ml 5 x 100ml 1 x 500ml
Trypsin-EDTA Solution 1X	TCL033-5X100ML TCL033-2X500ML TCL033-6X500ML	5 x 100ml 2 x 500ml 6 x 500ml
Trypsin Inhibitor from soybean 1X; Liquid	TCL068-1X100ML TCL068-5X100ML	1 x 100ml 5 x 100ml
Dulbecco's Phosphate Buffered Saline	TL1006-5X100ML TL1006-2X500ML TL1006-6X500ML TL1006-18X500ML TL1006-1X1000ML	5 x 100ml 2 x 500ml 6 x 500ml 18 x 500ml 1 x 1000ml
Antibiotic Antimycotic solution 100X, Liquid	A002-5X20ML A002-5X50ML A002-5X100ML	5 x 20ml 5 x 50ml 5 x 100ml
Gentamycin Solution	A005-5X20ML A005-5X50ML	5 x 20ml 5 x 50ml

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