HiMedia CellCulture
Enabling Breakthroughs

Product Information

HiKeratinoXL™ Keratinocyte Expansion Medium, Serum free

Product Code: AL524

Product description:

HiKeratinoXL™ Keratinocyte Expansion Medium is a serum free medium used for *in vitro* cultivation and expansion of Human Adult Epidermal Keratinocytes (HAEK) and Human Epidermal Keratinocytes from Juvenile Foreskin. It contains basal medium (Part A), keratinocyte growth supplement (Part B) and Bovine Pituitary Extract (Part C). 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 keratinocytes. 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
3. Reagent for Coating Culture Vessel	Code
1% Collagen Solution in DPBS	TCL127

Directions:

1. Thaw keratinocyte growth supplement (Part B) overnight at 2-8ºC.
   Note: Precipitates in Part C after thawing are normal. Precipitates will not affect the performance of the medium.
2. Disinfect the external surface of the bottles of part A part B and part C by spraying with isopropyl alcohol before placing in a bisafety hood.
3. Transfer the entire content of part B and part C to basal medium (part A) under aseptic condition.
   Note: If desired, 5ml of antibiotic - antimycotic solution (A002) or 0.5ml of gentamicin - amphotericin B solution (A031) 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.

Quality control:

Appearance

Part A: Light pink coloured clear solution
Part B: Pale yellow coloured clear solution
Part C: Amber coloured hazy liquid

pH

7.00-7.60

Osmolality in mOsm/Kg H_2O

320.00-360.00

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 human keratinocytes.

Storage and shelf life:

Store basal medium at 2-8ºC away from bright light. Store keratinocyte growth supplement at -20 ºC. Use before expiry date given on the product label. Shelf life of the complete medium is 4 weeks at 2-8ºC.

Note: Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement and bovine pituitary extract.

Table 1 : Collagen Coating of Culture Vessel

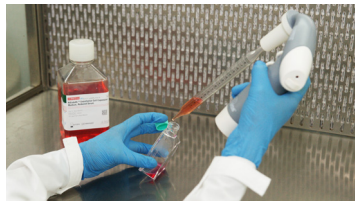
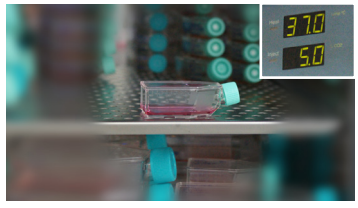
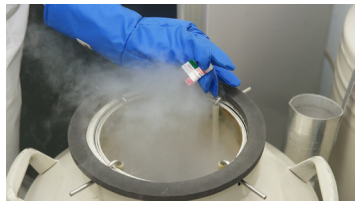
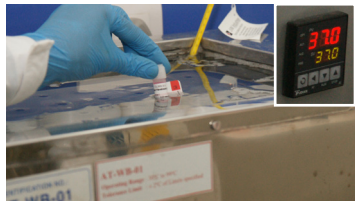
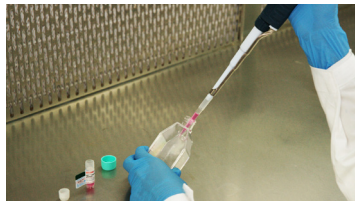
		Key Points to Remember	Time Required (approx.)
		For uniform coating , make sure that the incubator is properly leveled	
Aseptically add 1% collagen solution (TCL127)		**Refer Table 2 for recommended volumes of collagen solution**	1 min
Incubate for 2 hrs at 37^{o}C incubator			2 hrs
Aspirate collagen solution with the help of pipette			
COLLAGEN COATED CULTURE VESSEL IS READY FOR USE			
If the vessel is not to be used immediately, store at 2-8^{o}C upto one week.		**Flasks should be kept with caps tightlyclosed and plates should be sealed with parafilm during storage**	

Table 2 : Recommended Volumes of Collagen 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** **AL524 (Part A-500 ml) + (Part B-1.2 ml) + (Part C-2 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. 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

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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.

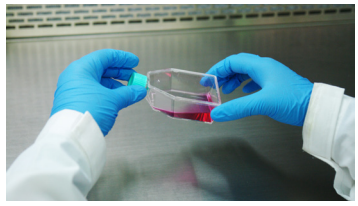
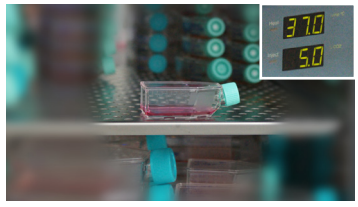
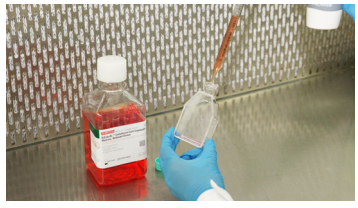
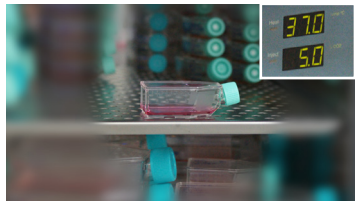
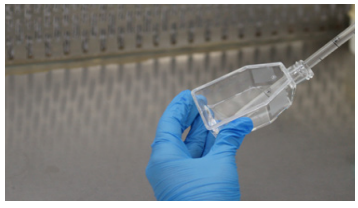
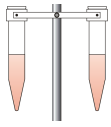
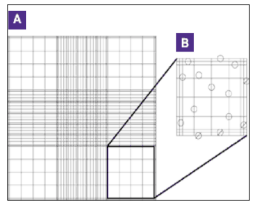
		Key Points to Remember	Time Required (approx.)
e. Cap the flask and shake gently to ensure proper mixing and uniform distribution of cells in the medium			10 secs
3. Incubation			
a. Incubate the cells at 37°C and 5% CO_2		**Check for cell attachment in 4-5 hrs** **On Day 1, cells may retain round shape and take morphology on Day 2 post revival**	2-3 hrs
b. If more than 70-80% cells are attached, replace the medium with fresh medium		**Medium change after 4-5 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** **DO NOT allow cells to reach 100% confluency before sub culture or cryopreservation.** **Upto 50% Confluency:** **Change the medium on alternate day** **After 50% Confluency:** **Change the medium everyday**	

Table 4 : Subculture

- HAEK/Human Epidermal Keratinocytes 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 HAEK 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 2 ml pre-warmed Trypsin-EDTA (TCL128) solution e. Incubate the flask at 37^{0}C for 1 min f. Check for rounding of the cells and keep tapping the flask gently for 1 min		**Gently rock the flask to ensure complete coverage of the Trypsin-EDTA solution over the cells** **Exposing the cells to Trypsin- EDTA for longer time leads to loss of cell viability** **This will allow complete dissociation of cells**	1 min 1 min
g. To neutralize action of trypsin add 4 ml of TCL068 h. Transfer contents of flask to 15 ml centrifuge tube using pipette i. Centrifuge the cells at 1000 rpm for 10 min		**Very small pellet will be observed**	15 secs 10 min
j. Carefully discard the supernatant by aspiration k. Add 2 ml of AL524 l. Resuspend the pellet by pipetting gently to get a homogenous mixture		**DO NOT POUR** **Vigorous pipetting will stress the cells**	60 secs
m. Count cells using hemocytometer n. 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

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- A confluent T-25 flask of HAEK yields 1.0 x 10^6 cells

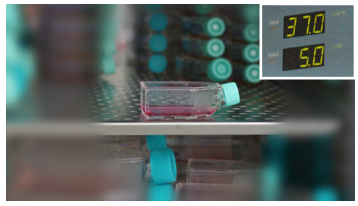
		Key Points to Remember	Time Required (approx.)
o. Incubate in a humidified incubator at 37°C and 5% CO_2			48 hrs
Maintenance			
a. Monitor the cells every day b. Change the medium c. Sub-culture once cells reach 70 - 80% confluence		**Upto 50% Confluency:** **Change the medium on alternate day** **After 50% Confluency:** **Change the medium everyday**	

Table 5 : Seeding Density

Flask	Recommended Seeding Densitiy	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 Adult Epidermal Keratinocytes (HAEK)	CL006-0.5 CL006-T25 CL006-T75	0.5 million cells/vial 1 T25cm^2 flask 1 T75cm^2 flask
HiFi™ Human Epidermal Keratinocytes from Juvenile Foreskin	CL009-0.5 CL009-T25 CL009-T75	0.5 million cells/vial 1 T25cm^2 flask 1 T75cm^2 flask
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
Gentamycin - Amphotericin B Solution 1000X	A031-5X20ML A031-5X50ML	5 x 20ml 5 x 50ml
Trypsin – EDTA Solution 1X w/ 0.025% Trypsin and 0.02% EDTA in Dulbecco's Phosphate Buffered Saline w/o Phenol red	TCL128-5X100ML TCL128-2X500ML TCL128-6X500ML	5 x 100ml 2 x 500ml 6 x 500ml

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