TECHNICAL DATA SHEET

YASHRAJ BIOTECHNOLOGY LTD.®
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Estd. 1999

Normal Human Epideimal Keratinocytes (NHEK)

Growth Serum-Free (SF) Medium

PRODUCT CODE

FcbKEM-226

PRODUCT DESCRIPTION

NHEK-Growth SF, 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, NHEK-growth supplement (Part A) and NHEK-Bovine Pituitary Extract (Part B). Basal medium consists of inorganic, organic salts, amino acids, vitamins and sodium bicarbonate. Part A consists of growth factors and nutrients necessary for growth of keratinocytes. This medium and the supplement is devoid of antibiotics and antimycotics.

Products required but not supplied by YBL

1. Media Supplements
Antibiotic-Antimycotic Solution 100X
Gentamicin-Amphotericin B solution 1000X
2. Reagents for Sub-culture
Dulbecco's Phosphate Buffered Saline (DPBS)
Trypsin-EDTA Solution 1X
Trypan Blue 0.5% Solution
Soyabean Trypsin Inhibitor
3. Reagent for Coating Culture Vessel
1% Collagen Peptide Solution in DPBS

DIRECTIONS

1. Thaw NHEK- Growth supplement (Part A) overnight at 2-8ºC.

   Note: *Precipitates in NHEK-Bovine Pituitary Supplement Part B after thawing are normal.*
   Precipitates will not affect the performance of the medium.

2. Disinfect the external surface of the bottles of Basal medium, NHEK Growth Supplement (Part A) and NHEK Bovine Pituitary Supplement (Part B) by spraying with 70% isopropyl alcohol before placing in a biosafety hood.

3. Transfer the entire content of Part A and Part B to basal medium under aseptic conditions.

   Note: *If desired, 5 ml of antibiotic - antimycotic solution* or 0.5 ml of gentamicin - amphotericin B solution can be added to 500 ml 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

Basal medium: Light pink coloured clear solution

Part A: Pale yellow coloured clear solution

Part B: 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 specifications.

Cultural Response

The medium is tested for optimal cell growth and proliferation of human keratinocytes.

STORAGE & SHELF LIFE

Store basal medium at 2-8ºC away from bright light. Store keratinocyte growth supplement at -20ºC. 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

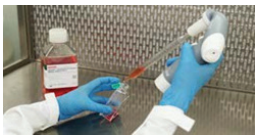
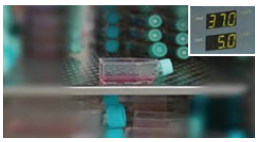
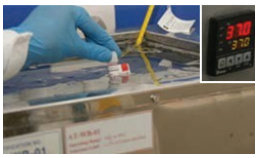
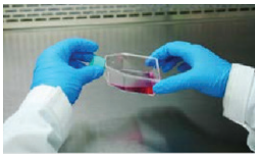
	Key Points to Remember	Time Required (approx.)
	For uniform coating, make sure that the incubator is properly levelled	
Aseptically add 1% collagen solution	Refer Table 2 for recommended volumes of collagen solution	1 min
Incubate for 2 hrs at 37°C incubator		2 hrs
Aspirate collagen solution with the help of pipette		
COLLAGEN COATED CULTURE VESSEL IS READY FOR USE		
If the vessel is not to be used immediately, store at 2-8°C upto one week.	Flasks should be kept with caps tightly closed and plates should be sealed with a 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 vapor shipper (-150ºC to -130ºC).
- 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.

Procedure		Key Points to Remember	Time Required (approx.)
1. Preparation of Culture Vessel			
a. Add 5 ml of complete medium to a T-25 flask.		**Preparation of complete medium** NHEK Growth Basal Medium + NHEK Growth Supplement (Part A) + NHEK Bovine Pituitary Supplement (Part B)	60 secs
b. Place the flask at 37°C to equilibrate the medium.			30 secs
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.		DO NOT centrifuge the cell suspension. Dropwise addition is required to prevent the cells from stress induced by exothermic reaction.	30-60 secs
e. Cap the flask and shake gently to ensure proper mixing and uniform distribution of cells in the medium.			10 secs

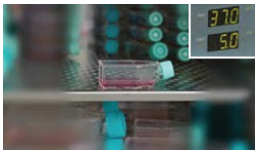
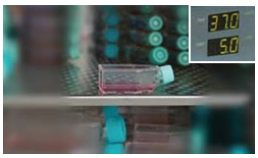
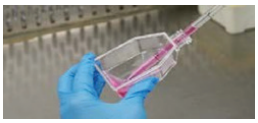
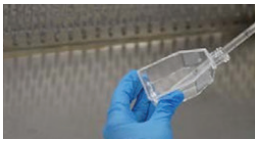
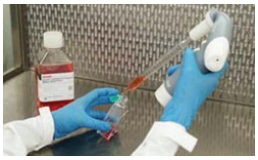
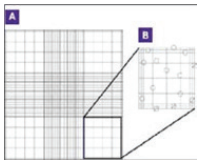
Procedure		Key Points to Remember	Time Required (approx.)
3. Incubation			
a. Incubate the cells at 37°C and 5% CO_2.		Check for cell attachment in 4-5 hrs. On day 1, cells may retain round shape and take morphology on day 2 post revival.	2-3 hrs
b. If more than 70-80% cells are attached, replace the 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
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 the 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 every day Use trypsin inhibitor solution for neutralization of Trypsin during subculture. Usage of complete medium for neutralization will result in inefficient neutralization and stress the cells resulting in reduced viability and cell death.	

TABLE 4 : SUB-CULTURE

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

Procedure		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 Aspirate off the DPBS and discard. c. Add 0.5 ml pre-warmed Trypsin-EDTA solution. d. Incubate the flask at 37°C for 30-60 secs.	 	Prior to use, make sure that Trypsin-EDTA solution is equilibrated to room temperature. 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.	60 secs 30-60 secs
e. Microscopically monitor the flask. f. When the cells start rounding up, gently tap the flask to ensure complete detachment of cells.			15 secs
g. If reduced serum medium AL528 is used, add 0.5 ml Soyabean Trypsin Inhibitor Solution h. Transfer the entire contents of the flask into a 15 ml centrifuge tube. i. Centrifuge the cell suspension at 1000 rpm for 10 mins. j. Discard supernatant and resuspend pellet in fresh 3 ml of complete medium by pipetting.		Vigorous pipetting will stress the cells.	60 secs
k. Count cells using hemocytometer. l. Seed at recommended seeding density in a new flask containing fresh complete media. Refer to Table 3 m. Incubate in a humidified incubator at 37ºC and 5% CO_2.	 	DO NOT refrigerate cells after splitting Seed immediately.	10-15 mins 48 hrs

Maintenance

- Monitor the cells every day.
- Change the medium every alternate day.
- Sub -culture once cells reach 70 - 80% confluence.

TABLE 5: SEEDING DENSITY

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

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