invitrogen PRODUCT INFORMATION SHEET

Human Epidermal Keratinocytes, neonatal (HEKn)

Catalog Number C-001-5C

Pub. No. MAN0001610

Rev. 3.0

Product description

HEKn are human epidermal keratinocytes isolated from neonatal foreskin. Each vial of this product contains ≥5 × 10^5 viable cells that have been cryopreserved at the end of the primary culture stage in a medium containing 10% DMSO. An independent laboratory tests the cells for the presence of mycoplasma, Hepatitis B, Hepatitis C, and HIV-1 viruses. These agents were not detected. In our laboratory, each lot of cells is performance tested by culturing the cells through multiple passages in EpiLife™ Medium supplemented with Human Keratinocyte Growth Supplement (HKGS) in the absence of antibiotics and antimycotics. During this culture period, no contamination by bacteria, yeast, or other fungi was detected. Upon thawing, the cells are guaranteed to be ≥70% viable (trypan blue) and to have a potential of ≥30 population doublings when handled according to the directions provided in this document. For recommended precautions for handling human cells, read the Caution statement.

Product	Catalog No.	Amount	Shipping	Storage
Human Epidermal Keratinocytes, neonatal (HEKn)	C-001-5C	1 vial (≥5 × 10^5 viable cells/vial)	Frozen on dry ice	Liquid nitrogen vapor phase

Intended use

Cryopreserved HEKn are intended for use by researchers investigating the molecular and biochemical bases of various normal and disease processes. **This product is for research use only. Not for use in animals, humans, or diagnostic procedures.**

Storage and stability

Cryopreserved HEKn should arrive frozen on dry ice. If the cells are not to be used immediately, prepare a space for storage of the vial in the vapor phase of a liquid nitrogen freezer. While wearing protective eyewear, gloves, and a laboratory coat, remove the vial from its shipping container and place immediately in the liquid nitrogen freezer. Although the viability of cryopreserved cells decreases with time in storage, useful cultures can usually be established even after 2 years of storage at liquid nitrogen temperatures

Caution

Although cryopreserved cells have been tested for the presence of various hazardous agents, diagnostic tests are not necessarily 100% accurate. In addition, human cells may harbor other known or unknown agents, or organisms which could be harmful to your health or cause fatal illness. Treat all human cells as potential pathogens. Wear protective clothing and eyewear. Practice appropriate disposal techniques for potentially pathogenic or biohazardous materials. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Required materials not supplied

	Standard system	Extended-lifespan systems	
	Undefined	Undefined	Defined
Basal Medium	Medium 154 (Cat. No. M154500)	EpiLife™ Medium (Cat. No. MEPI500CA)	EpiLife™ Medium (Cat. No. MEPI500CA)
Growth Supplement	HKGS (Cat. No. S-001-5) or HKGS Kit (Cat. No. S-001-K)	HKGS (Cat. No. S-001-5) or HKGS Kit (Cat. No. S-001-K)	EDGS [1] (Cat. No. S-012-5)
Coating Matrix Kit	N/A	N/A	Coating Matrix Kit (Cat. No. R-011-K)
Subculture Reagent	Trypsin/EDTA (Cat. No. R-001-100)	Trypsin/EDTA (Cat. No. R-001-100)	Trypsin/EDTA (Cat. No. R-001-100)
Subculture Reagent	Trypsin Neutralizer (Cat. No. R-002-100)	Trypsin Neutralizer (Cat. No. R-002-100)	Defined Trypsin Inhibitor (Cat. No. R-007-100)

[1] For optimal cell growth, EDGS should be used in conjunction with Coating Matrix Kit and Defined Trypsin Inhibitor.

Expected lifespan of keratinocyte cultures

	Standard system	Extended-lifespan systems	
	Undefined	Undefined	Defined
Human Epidermal Keratinocytes, neonatal (HEKn) (Cat. No. C-001-5C)	16–25 population doublings	50–70 population doublings	40–60 population doublings
Human Epidermal Keratinocytes, adult (HEKa) (Cat. No. C-005-5C)	16–20 population doublings	35–45 population doublings	30–40 population doublings

Initiate cultures from cryopreserved cells

We recommend seeding cells recovered from cryopreservation at a density of 2.5×10^3 viable cells/cm^2. For example, three 75-cm^2 or nine 25-cm^2 tissue culture flasks can usually be established from one vial containing $\geq 5 \times 10^5$ HEKn. The following procedure is a sample protocol for establishing cultures from the contents of one vial.

1. From the tables above, determine which basal medium and growth supplement you wish to use, then prepare a bottle of supplemented medium according to the instructions that accompany that product.
2. Remove the vial of cells to be thawed from liquid nitrogen and rapidly thaw by placing at 37°C in a water bath with gentle agitation for 1–2 minutes (or once a sliver of ice is left in the tube). Complete thawing can be detrimental to the cell viability.
3. When the contents of the vial have thawed, wipe the outside of the vial with disinfecting solution and move to a Class II, type A laminar flow culture hood.
4. Open the vial and pipette the suspension up and down with a 1-mL pipette to disperse the cells.
5. Remove 20 µL from the vial and dilute the cell suspension in 20 µL of trypan blue solution (for example, Invitrogen™ Trypan Blue Solution, 0.4%, Cat. No. 15250-061).
6. Use a hemacytometer to determine the number of viable cells per mL.
7. Dilute the contents of the vial (1 mL) to a concentration of 1.25×10^4 viable cells/mL using the supplemented medium from step 1, above.
8. Add 5 mL of cell suspension to each 25-cm^2 culture flask or 15 mL of cell suspension to each 75-cm^2 culture flask.
9. Following inoculation, swirl the medium in the flasks to distribute the cells. HEKn attach to culture surfaces quickly, and if the medium is not distributed immediately following inoculation, the cells can grow in uneven patterns.
10. Incubate the cultures in a 37°C, 5% CO_2/95% air, humidified cell culture incubator. For best results, do not disturb the culture for at least 24 hours after the culture has been initiated.

Maintain stock cultures

1. Change the culture medium to freshly supplemented medium, 24 to 36 hours after establishing a secondary culture from cryopreserved cells. For subsequent subcultures, change the medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change the medium every day until the culture is approximately 80% confluent.

Notes

- To achieve the highest cell densities, the culture medium should be changed every day as the cultures approach confluence. To obtain rapidly proliferating subcultures, HEKn should be subcultured before they become more than 80% confluent. If HEKn reach confluence, the cells mitotically arrest and some of the cells leave the proliferating pool. Allowing HEKn cultures to arrest decreases the long-term potential yield from a cryopreserved vial. The number of subcultures (passages) that can be achieved varies with the starting cell density and the methods employed.
- HEKn cultures seeded at 2.5×10^3 cells/cm^2 from cryopreserved cells should reach 80% confluence in 5–7 days. In this culture, most of the cells should have an epithelioid morphology and be associated with each other in colonies. Some irregularly sized and shaped cells may be observed. Occasionally, small numbers of melanocytes persist in the secondary culture. Melanocytes do not readily proliferate in medium supplemented with HKGS or EDGS, and should be virtually absent in subsequent cultures.

Subculture HEKn

View the culture under the microscope to confirm that it is subconfluent, and that there are mitotic cells present. This protocol is designed for the subculture of one 25-cm^2 culture flask. If different-sized culture vessels are to be used, adjust reagent volumes accordingly.

1. Assemble the appropriate supplemented medium and subculture reagents according to the instructions that accompany the products.

Note: We do **NOT** recommend warming the reagents prior to use.

2. Assemble the appropriate culture vessels, sterile pipettes, and sterile 15-mL conical tubes (not provided).
3. Remove all of the culture medium from the flask.
4. Add 3 mL of Trypsin/EDTA solution to the flask. Rock the flask to ensure that the entire surface is covered.
5. Immediately remove all 3 mL of Trypsin/EDTA solution from the flask.
6. Add 1 mL of fresh Trypsin/EDTA solution to the flask.
7. View the culture under a microscope. Incubate the flask at room temperature until the cells have become completely round, approximately 8–10 minutes.
8. Rap the flask very gently to dislodge cells from the surface of the flask.
9. Add 3 mL of Trypsin Neutralizer solution or Defined Trypsin Inhibitor solution to the flask and transfer the detached cells to a sterile 15-mL conical tube.
10. Add 3 mL additional Trypsin Neutralizer solution or Defined Trypsin Inhibitor solution to the flask and pipette the solution over the flask surface several times to remove any remaining cells. Add this solution to the 15-mL conical tube.
11. Centrifuge the cells at $180 \times g$ for 7 minutes. Observe the cell pellet.
12. Remove the supernatant from the tube, being careful not to dislodge the cell pellet.
13. Resuspend the cell pellet in 4 mL supplemented medium. Pipette the cells up and down with a 10-mL pipette to ensure a homogeneous cell suspension.
14. Determine the concentration of cells in the suspension.
15. Dilute the cells in supplemented medium and seed new culture vessels with 2.5×10^3 cells/cm^2.
16. Incubate the cultures in a 37°C, 5% CO_2/95% air, humidified cell culture incubator.

Notes

- Damage to cultured HEKn can occur during trypsinization. This damage can result from exposure of the cells to the Trypsin/EDTA solution for excessive lengths of time, trypsinization at temperatures exceeding room temperature, and/or excessive mechanical agitation. Check to make sure that the temperature of trypsinization is appropriate and, if necessary, alter the incubation time of the procedure.
- Another common source of damage is centrifugation at excessive *g* forces. Check to make sure that the speed of the centrifuge is appropriate. One manifestation of cellular damage that may be evident after centrifugation is strings of cells (and debris) that do not pellet at the bottom of the tube. This is due to the presence of DNA from lysed cells in the solution. If this condition exists, the cell pellet can be lost upon aspiration of the supernatant containing the DNA strings. In many cases, viable cells can be rescued by pipetting the cells (and DNA) up and down with a 10-mL pipette to shear the DNA, and centrifuging the suspension again to recover the cells.

Limited product warranty

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The information in this guide is subject to change without notice.