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Human Epidermal Keratinocytes, pooled (HEKn pooled)

Catalog no. A13401

Quantity: ≥1 × 10^6 viable cells/vial

Product Description

HEKn pooled are human epidermal keratinocytes isolated from multiple neonatal foreskins. Each vial of this product contains ≥1 × 10^6 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 ≥16 population doublings when handled according to the directions provided in this document. For recommended precautions to be taken when handling human cells, please read the caution statement.

Intended Use

Cryopreserved HEKn pooled 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 intended for human or animal therapeutic or diagnostic use.**

Storage and Stability

Cryopreserved HEKn pooled should arrive frozen on dry ice. If the cells are not to be used immediately, the user should 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. The user should treat all human cells as potential pathogens. Wear protective clothing and eyewear. Practice appropriate disposal techniques for potentially pathogenic or biohazardous materials.

Initiating Cultures from Cryopreserved Cells

Seed cells recovered from cryopreservation at a density of 2.5 × 10^3 viable cells/cm^2. For example, six 75 cm^2 or three 150 cm^2 tissue culture flasks can usually be established from one vial containing ≥1 × 10^6 HEKn pooled. The procedure given below is a sample protocol for establishing cultures from the contents of one vial.

1. From the table on page 2, determine which basal medium and growth supplement you wish to use and prepare a bottle of supplemented medium according to the instructions that accompany that product.
2. Remove a vial of HEKn pooled from liquid nitrogen storage, taking care to protect hands and eyes.
3. Dip the lower half of the vial into a 37°C water bath to thaw (approximately 2–3 minutes).
4. 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.
5. Open the vial and pipette the suspension up and down with a 1 mL pipette to disperse the cells.
6. Remove 20 µL from the vial and dilute the cell suspension in 20 µL of trypan blue solution (Cat. no. 15250-061).
7. Use a hemacytometer to determine the number of viable cells/ mL.
8. Dilute the contents of the vial (1 mL) to a density of 1.25 × 10^4 viable.
9. Add 15 mL of cell suspension to each 75 cm^2 culture flask or 30 mL of cell suspension to each 150 cm^2 culture flask.
10. Following inoculation, swirl the medium in the flasks to distribute the cells. HEKn pooled attach to culture surfaces quickly, and if the medium is not distributed immediately following inoculation, the cells may grow in uneven patterns.
11. 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.

Keratinocyte Culture Systems from Gibco®		
	Optimized for HEKn pooled	
	Undefined	**Defined and Animal Origin Free (AOF)**
Basal Medium	EpiLife® Medium (M-EPI-500-CA)	EpiLife® Medium (M-EPI-500-CA)
Growth Supplement	HKGS (S-001-5) *or* HKGS Kit (S-001-K)	S7 (S-0175)
Coating Matrix Kit	N/A	Coating Matrix Kit (R-011-K)
	Compatible with HEKn pooled	
	Undefined	**Defined**
Medium kit	Keratinocyte Serum Free Medium (17005-042)	Defined Keratinocyte Serum Free Medium (10744-019)
Coating Matrix Kit	N/A	Coating Matrix Kit (R-011-K)
Related Reagents		
	Undefined	**Defined and Animal Origin Free (AOF)**
Subculture Reagent	Trypsin/EDTA (R-001-100)	TrypLE™ Select (12563-011)
Subculture Reagent	Trypsin Neutralizer (R-002-100)	
1.Gibco® recommends EpiLife® medium systems for optimal cell growth, although Keratinocyte Serum Free Medium systems support growth of ≥16 population doublings. 2. Coating Matrix Kit is required for use with either Defined growth system.		

Maintenance of 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 pooled should be subcultured before they become more than 80% confluent. If HEKn pooled reach confluency, the cells mitotically arrest and some of the cells leave the proliferating population. Allowing HEKn pooled cultures to arrest will decrease the long-term potential yield from a cryopreserved vial. The number of subcultures (passages) that can be achieved will vary with the starting cell density and the methods employed by individual investigators.
- HEKn pooled 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 HEK medium, and should be virtually absent in subsequent cultures.

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Subculture of HEKn pooled

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 75 cm^2 culture flask. If different-sized culture vessels are to be used, reagent volumes should be adjusted accordingly.

1. Assemble the appropriate supplemented medium and subculture reagents according to the table on page 2.
2. Assemble the appropriate culture vessels, sterile pipettes, and sterile 50 mL conical tubes (not provided).
3. Remove all of the culture medium from the flask.
4. Add 9 mL of Trypsin/EDTA solution to the flask. Rock the flask to ensure that the entire surface is covered.
5. Immediately remove all 9 mL of Trypsin/EDTA solution from the flask.
6. Add 3 mL of fresh Trypsin/EDTA solution to the flask, swirl gently to ensure even distribution.
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 9 mL of Trypsin Neutralizer solution or Defined Trypsin Inhibitor solution to the flask and transfer the detached cells to a sterile 50 mL conical tube.
10. Add 9 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 50 mL conical tube.
11. Centrifuge the cells at 180 × *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 12 mL supplemented medium. Pipette the cells up and down with a 10 mL pipette to ensure a homogeneous cell suspension.
14. Determine the density 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

- TrypLE™ (trypsin-like enzyme) may be substituted for Trypsin/EDTA (used above in steps 4–6), neutralization of TrypLE™ following dislodging of cells (step 9 above) should be done using complete culture medium (e.g., EpiLife® with HKGS)
- Damage to cultured HEKn pooled can occur during trypsinization. This damage may 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.
- 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 in the bottom of the tube. This is due to the presence of DNA from lysed cells in the solution. If In many cases, viable cells can be rescued by pipetting the cells (and DNA) up and down in a 10 mL pipette to shear the DNA, and centrifuging the suspension again to recover the cells.

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