



Human Epidermal Keratinocytes (neonatal)

“Animal Product-Free”

HEKn-APF

Catalog Number: C-020-5C

≥ 500,000 viable cells/vial

Instructions for storage, initiation of cultures from cryopreserved cells, and subculture

Product Description

HEKn-APF are human epidermal keratinocytes from neonatal foreskin that are isolated, grown, and cryopreserved in an environment free of animal-derived components (“animal product-free”, or “APF”). Each vial of this product contains $\geq 5 \times 10^5$ viable cells that have been cryopreserved at the end of the primary culture stage in Synth-a-Freeze® Cryopreservation Medium. 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-V2 (HKGS-V2) 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 $\geq 70\%$ viable and to have a potential of ≥ 30 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 box under “Initiating cultures from cryopreserved cells.”

Intended Use

Cryopreserved HEKn-APF 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-APF 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.

Initiating Cultures from Cryopreserved Cells

Caution: Although cryopreserved cells from Cascade Biologics, Inc. 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.

We recommend seeding cells recovered from cryopreservation at a density of 2.5×10^3 viable cells/cm² on tissue culture plastic that has been coated with Cascade Biologics' Coating Matrix Kit (cat. # R-011-K). For example, three 75 cm² or nine 25 cm² tissue culture flasks can usually be established from one vial containing $\geq 5 \times 10^5$ HEKn-APF. The procedure given below is a sample protocol for establishing cultures from the contents of one vial.

Note: HEKn-APF can be serially propagated in EpiLife Medium supplemented with HKGS-V2 using untreated tissue culture labware. However, using tissue culture labware treated with Coating Matrix Kit will enhance growth rates and culture longevity.

- 1) If desired, treat the appropriate number and type of culture vessels with Cascade Biologics' Coating Matrix Kit (cat. # R-011-K) using aseptic technique as follows:
 - a) Add Dilution Medium (cat. #R-012-50) to each flask (5 ml per each 75 cm² flask, or 1.7 ml per each 25 cm² flask).
 - b) Add Coating Matrix (cat. #R-011-05) directly to the Dilution Medium in each flask (50 μ l per each 75 cm² flask, or 17 μ l per each 25 cm² flask). Swirl gently.
 - c) Cap the flasks and incubate for 30 minutes at room temperature.
 - d) Remove excess Coating Matrix/Dilution Medium from each flask. The flasks may be used immediately, or may be stored at 4 °C for short periods.
- 2) Prepare a bottle of supplemented EpiLife Medium (cat. # M-EPI-500-CA) according to the instructions that accompany that product.
- 3) Remove a vial of HEKn-APF from liquid nitrogen storage, taking care to protect hands and eyes.
- 4) Dip the lower half of the vial into a 37 °C water bath to thaw.
- 5) 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.
- 6) Open the vial and pipette the suspension up and down with a 1 ml pipette to disperse the cells.

- 7) Remove 20 μ l from the vial and dilute the cell suspension in 20 μ l of trypan blue solution (for example: Sigma Chemical Company's cat. #T8154).
- 8) Use a hemacytometer to determine the number of viable cells per ml.
- 9) 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 2, above.
- 10) Add 5 ml of cell suspension to each 25 cm² culture flask or 15 ml of cell suspension to each 75 cm² culture flask that has been previously coated with Cascade Biologics' Coating Matrix Kit.
- 11) Following inoculation, swirl the medium in the flasks to distribute the cells. HEKn-APF attach to culture surfaces quickly, and if the medium is not distributed immediately following inoculation, the cells may grow in uneven patterns.
- 12) Incubate the cultures in a 37°C, 5% CO₂/95% air, humidified cell culture incubator. For best results, do not disturb the culture for at least 24 hours after the culture has been initiated.

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-APF should be subcultured before they become more than 80% confluent. If HEKn-APF reach confluence, the cells mitotically arrest and some of the cells leave the proliferating pool. Allowing HEKn-APF 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-APF cultures seeded at 2.5×10^3 cells/cm² from cryopreserved cells should reach 80% confluence in 4-6 days. In this culture, most of the cells should have an epithelioid morphology. 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-V2, and should be virtually absent in subsequent cultures.

Subculture of HEKn-APF

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 cm² culture flask. If different-sized culture vessels are to be used, reagent volumes should be adjusted accordingly.

- 1) Assemble subculture reagents and materials:
 - EpiLife Medium supplemented with HKGS-V2
 - Recombinant Trypsin/EDTA solution (cat. # R-009-50)
 - Defined Trypsin Inhibitor (cat. # R-007-100)
 - Culture vessels (not provided) coated with Cascade Biologics Coating Matrix Kit (cat. # R-011-K)
 - Sterile pipettes (not provided)
 - Sterile 15 ml conical tubes (not provided)

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 2 ml of recombinant Trypsin/EDTA solution to the flask. Rock the flask to ensure that the entire surface is covered.
- 5) View the culture under a microscope. Incubate the flask at room temperature until the cells have become completely round, approximately 8-10 minutes (15-18 minutes if labware has been treated with Coating Matrix Kit).
- 6) Rap the flask very gently to dislodge cells from the surface of the flask.
- 7) Add 3 ml of Defined Trypsin Inhibitor solution to the flask and transfer the detached cells to a sterile 15 ml conical tube.
- 8) Add 3 ml additional 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.
- 9) Centrifuge the cells at 180 x g for 7 minutes. Observe the cell pellet.
- 10) Remove the supernatant from the tube, being careful not to dislodge the cell pellet.
- 11) 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.
- 12) Determine the concentration of cells in the suspension.
- 13) Dilute the cells in supplemented medium and seed new culture vessels with 2.5×10^3 cells/cm².
- 14) Incubate the cultures in a 37°C, 5% CO₂/95% air, humidified cell culture incubator.

Note: Damage to cultured HEKn-APF can occur during trypsinization. This damage may result from exposure of the cells to the Recombinant 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 in 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 may 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 in a 10 ml pipette to shear the DNA, and centrifuging the suspension again to recover the cells.

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