Cascade Biologics™
invitrogen cell culture

GIBCO®
invitrogen cell culture

Human Dermal Fibroblasts, neonatal (HDFn)

Cat. no. C-004-5C

Quantity: ≥500,000 viable cells/vial

Product Description

Human dermal fibroblasts (HDFn) are human dermal fibroblasts isolated from neonatal foreskin. Each vial of this product contains ≥5 x 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 Medium 106 supplemented with Low Serum Growth Supplement (LSGS) in the absence of antibiotics and antimycotics. During this culture period, no contamination by bacteria, yeast, or fungi was detected. Upon thawing, the cells are guaranteed to be ≥70% viable 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 HDFn 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 HDFn 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

We recommend seeding cells recovered from cryopreservation at a density of 2.5 x 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 ≥5 x 10^5 HDFn. The procedure given below is a sample protocol for establishing cultures from the contents of one vial.

1. Prepare a bottle of supplemented Medium 106 (Cat. no. M-106-500) according to the instructions that accompany that product.
2. Remove a vial of HDFn from liquid nitrogen storage, taking care to protect hands and eyes.
3. Dip the lower half of the vial into a 37ºC water bath to thaw.
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 (for example: Sigma Chemical Company's Cat. no.T8154).
7. Using a hemacytometer, determine the number of viable cells per ml.
8. Dilute the contents of the vial (1 ml) to a concentration of 1.25 x 10^4 viable cells/ml using the supplemented medium from step 1, above.
10. 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.
11. Following inoculation, swirl the medium in the flasks to distribute the cells. HDFn 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 37ºC, 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.

Maintenance of Stock Cultures

1. Change the culture medium to freshly supplemented Medium 106, 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 80% confluent.
3. Once the culture reaches 80% confluency, change the medium every day.

Notes

- To achieve the highest cell densities, the culture medium should be changed every day as the cultures approach confluency. For rapidly proliferating subcultures, HDFn should be subcultured before the culture becomes confluent. However, HDFn can be maintained at confluence for 1-2 weeks and subsequently subcultured successfully. The number of subcultures (passages) that can be achieved will vary with the starting cell density and the methods employed by individual investigators.
- HDFn cultures seeded at 2.5 x 10^3 cells/cm^2 from cryopreserved cells should reach 80% confluency in 5-6 days. In this culture, most of the cells should have a bipolar morphology, and be distributed initially as individual cells. Some irregularly sized and shaped cells may be observed.

Subculture of HDFn

View the culture under a microscope to ascertain the condition of the culture (i.e., confluence, mitotic activity). This protocol is designed for the subculture of one 25 cm^2 culture flask of actively proliferating cells near confluence. If different-sized culture vessels are to be used, reagent volumes should be adjusted accordingly.

1. Assemble subculture reagents and materials:
   - Medium 106 supplemented with LSGS or LSGS Kit
   - Trypsin/EDTA solution (Cat. no. R-001-100)
   - Trypsin Neutralizer solution (Cat. no. R-002-100)
   - Culture vessels (not provided)
   - Sterile pipettes (not provided)
   - Sterile 15 ml conical tubes (not provided)

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

2. Remove all of the culture medium from the flask.
3. Add 4 ml of Trypsin/EDTA solution to the flask. Rock the flask to ensure that the entire surface is covered.
4. Immediately remove 3 ml of Trypsin/EDTA solution from the flask.
5. Incubate the flask at room temperature for 1-3 minutes.
6. View the culture under a microscope.
7. When the cells have become partially detached and rounded, rap the flask gently to dislodge the cells from the surface of the flask.
8. Add 3 ml of Trypsin Neutralizer to the flask and transfer the detached cells to a sterile 15 ml conical tube.
9. Add 3 ml additional Trypsin Neutralizer 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.
10. Centrifuge the cells at 180 x g for 7 minutes. Observe the cell pellet.
11. Remove the supernatant from the tube, being careful not to dislodge the cell pellet.
12. Resuspend the cell pellet in 4 ml supplemented Medium 106. Pipette the cells up and down with a 10 ml pipette to ensure a homogeneous cell suspension.
13. Determine the concentration of cells in the suspension.
14. Dilute the cells in supplemented Medium 106 and seed new culture vessels with 2.5 x 10^3 cells/cm^2.
15. Incubate the cultures in a 37°C, 5% CO_2/95% air, humidified cell culture incubator.

Notes

- Damage to cultured HDFn 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. 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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