Product Description

Normal Human Dermal Fibroblasts (NHDFs) are isolated from the dermis of neonatal foreskin (NHDF-CRY-NEO) or adult (breast or abdominal) skin (NHEK-CRY-AD) and cryopreserved. NHDFs exhibit spindle-like morphology in monolayer culture (Figure 1) and are characterized by positive staining for the fibroblast specific marker, vimentin (Figure 2). NHDFs are guaranteed to provide a minimum of 15 population doublings when handled according to the protocol below. Cells test negative for mycoplasma, bacteria, yeast, other fungi, Hepatitis B, Hepatitis C, and HIV-1.

Intended Use

MatTek's NHDFs provide an ideal culture system to study extracellular matrix proteins, wound healing, collagen production, disorders of fibroblasts such as fibrosis, and dermal toxicity. NHDFs require the use of DMEM-10 for optimal expansion in monolayer culture.

This product is for research use only. It is not intended for human or animal therapeutic or diagnostic use.

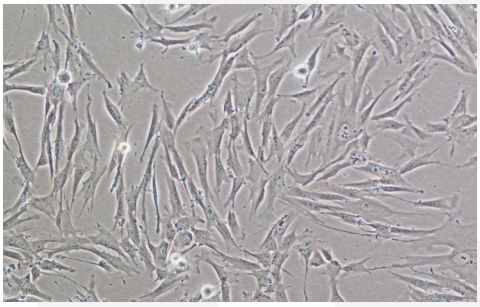

Figure 1. Phase contrast image of NHSF cells in monolayer culture, 100X total magnification.

Format

NHDFs are provided frozen in 1 mL of cryopreservation medium. Each vial contains ≥500K viable cells.

Storage & Handling

Upon receipt, transfer the cryovial(s) immediately into the vapor phase of a liquid nitrogen storage dewar. Cells are stable for more than two years when stored under these conditions. See Table 1 for reagent storage conditions.

Directions for Use

DMEM-10 (Dulbecco's Modified Eagle's Medium containing 10% fetal calf serum) is recommended for use with NHDFs.

Note: Antibiotics are not required, however they may be added. If desired, up to 1000U/mL Penicillin-Streptomycin (Gibco cat# 15140122) may be used without compromising cell growth or function.

Thawing and Seeding of Cryopreserved Cells

1. The recommended seeding density for NHDFs is 3500 cells/cm^2.
2. Aseptically vent any nitrogen from the cryovial in a biosafety cabinet prior to thawing. Quickly thaw the cryovial in a 37°C water bath, being careful not to submerge the cap. Watch the cryovial closely; when the last sliver of ice remains, remove the vial and wipe with 70% ethanol before opening in a biosafety cabinet.
3. Transfer cells from the cryovial(s) into a sterile conical tube containing into a sterile conical tube containing an appropriate volume of room temperature DMEM-10 for the cell culture vessel you are using. Cap and gently swirl to ensure even distribution of cells.
4. Gently transfer the cell suspension into the culture vessel(s). Carefully rock the culture vessel(s) to evenly distribute the cells and place in a 37°C, 5% CO_2, humidified incubator.

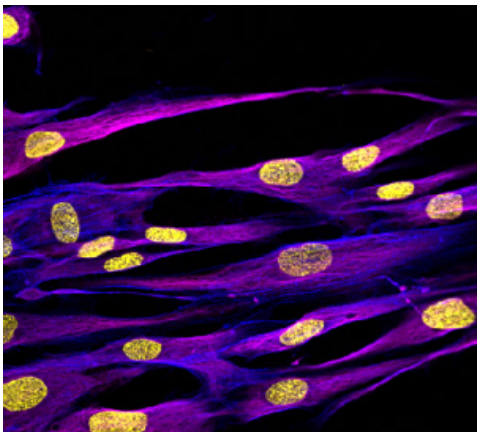

Figure 2. Immunofluorescent characterization of NHDFs showing vimentin (purple), phalloidin (blue) and DAPI (yellow) expression, 600X.

Maintenance of Monolayer Cell Cultures

1. Change the DMEM-10 growth medium 24 hours after initial plating and every 48 hours thereafter using 1 mL of pre-warmed media for every 5 cm^2 culture area.
2. Avoid repeated warming and cooling of the DMEM-10.

Subculturing of Cells

1. Subculture the cells when they are 70-80% confluent and contain many mitotic figures (generally 5-7 days after plating).
2. Prepare sufficient Trypsin (0.025%)/EDTA (0.265 mM) solution in Ca^{2+}/Mg^{2+}-free PBS (DPBS) in DPBS at room temperature (0.165 mL per 5 cm^2 of culture surface area).
3. Aspirate the medium from the culture vessel and rinse twice with DPBS (0.33 mL per 5 cm^2).
4. Add Trypsin/EDTA solution (0.165 mL per 5 cm^2) and gently rock to ensure complete coverage of cells. Incubate at room temperature for approximately 5 min or until cells completely round up.
5. Continue trypsinization until most cells (~80%) can be detached by gentle agitation of the vessel. Once ~80% of the cells have detached, rap the vessel against the edge of a hard surface 3-4 times to release remaining cells. To avoid damage to the cells, care should be taken to keep the trypsin exposure to the minimum time necessary.
6. After the cells have detached, add DMEM-10 (0.33 mL / 5cm^2) to the vessel and collect the cells in a conical tube.
7. Rinse the culture vessel with DPBS (0.33 mL / 5 cm^2) and transfer the rinse solution to the conical tube containing the cell suspension.
8. Take a small aliquot for cell counting and centrifuge the remaining suspension at 100 X g for 5 minutes.
9. Resuspend the pellet in fresh, warm DMEM-10. Seed cells into a new culture vessel at a density of 3500 cells/cm^2.

Table 1. NHDF Ordering Information

Product	Catalog Number	Size	Storage
Normal Human Dermal Fibroblasts (Neonatal)	NHDF-CRY-NEO	≥5x10^5 cells (1 mL)	Liquid Nitrogen Vapor
Normal Human Dermal Fibroblasts (Adult)	NHDF-CRY-AD		

MatTek

200 Homer Ave
Ashland, MA 01721
USA
+1-508-881-6771

Mlynske Nivy 73
Bratislava, 821 05
Slovak Republic
+421-2-3260-7401

support@mattek.com
www.mattek.com