MatTek CORPORATION

Human Bronchial Fibroblasts Data Sheet

Product Description

Normal Human Bronchial Fibroblasts (NHBFs) exhibit spindle-like morphology in monolayer culture (**Figure 1**) and are characterized by positive staining for the fibroblast marker, vimentin (**Figure 2**). Cells are guaranteed to provide a minimum of 15 population doublings when handled according to the protocol below. Cells test negative for Hepatitis B, Hepatitis C, HIV-1, mycoplasma, bacteria, yeast, and fungi.

Intended Use

NHBFs require the use of DMEM-10 for optimal expansion in monolayer culture. When used with DMEM-10, NHBFs provide an ideal culture system to study extracellular matrix production, wound healing and fibrosis.

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

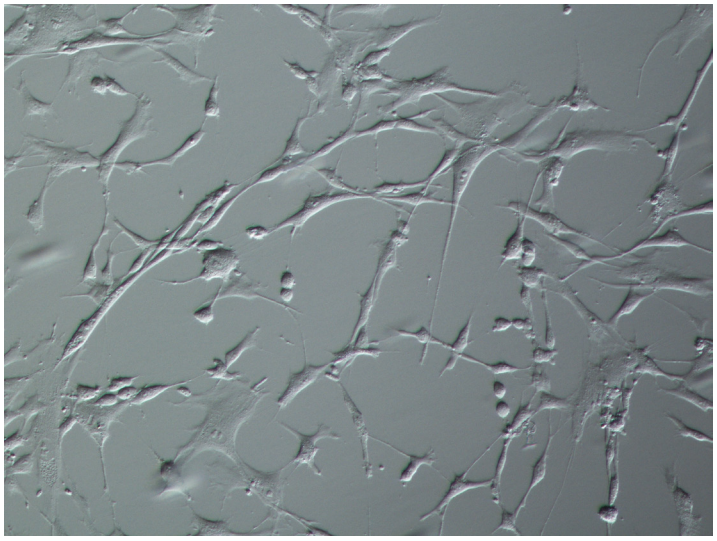

Figure 1. Phase contrast image of NHBFs in monolayer culture, 100X.

Format

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

Storage & Handling

Upon receipt, immediately transfer the cryovial(s) into the vapor phase of a liquid nitrogen storage dewar. Cells are stable for ≥ 2 years when stored under these conditions. See **Table 1** for cell 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 NHBFs is 3500 cells/cm^2.
2. Aseptically vent any nitrogen from cryovial in a biosafety cabinet prior to thawing by briefly loosening and re-tightening the cap. 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 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.

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 growth medium.

Table 1. NHBF Product Information			
Product	Catalog Number	Size	Storage
Normal Human Bronchial Fibroblasts (Non-Diseased)	NHBF-CRY	≥5x10^5 cells (1 mL)	Liquid Nitrogen Vapor
Normal Human Bronchial Fibroblasts (Diseased)	NHBF-CRY-DS		

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) at room temperature (0.165 mL per 5 cm^2 culture area).
3. Aspirate the medium from the culture vessel and rinse twice with DPBS (0.33 mL/5 cm^2).
4. Add Trypsin/EDTA solution (0.165 mL/5 cm^2 culture area) and gently rock to ensure complete coverage. Incubate at room temperature for 5 min or until cells completely round up.
5. Continue trypsinization until ~80% of the cells can be detached by gentle agitation of the vessel. Then, rap the vessel against a hard surface 3-4 times to release remaining cells. To avoid damage to the cells, keep the trypsin exposure to the minimum time necessary.
6. After the cells have been detached, add DMEM-10 (0.33 mL/5cm^2 culture area) to the vessel and collect the cells in a conical tube.
7. Rinse the culture vessel with DPBS (0.33 mL/5 cm^2 culture area) and combine with 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 3,500 cells/cm^2.

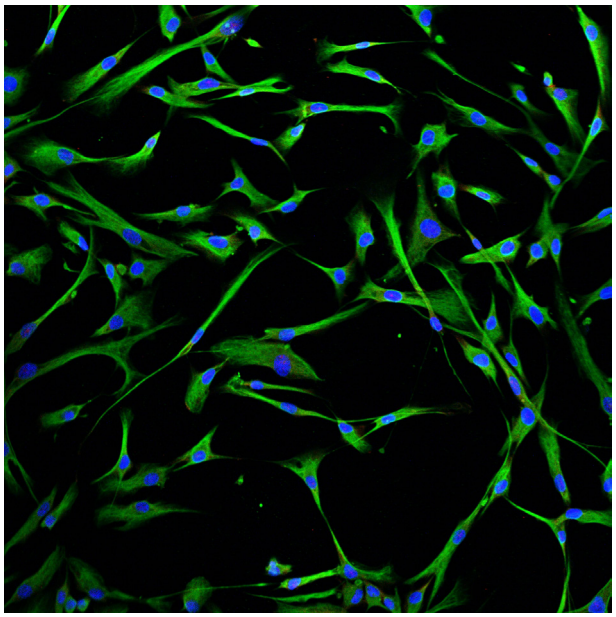

Figure 2. Immunofluorescent characterization of NHBFs showing DAPI (blue), and vimentin (green) expression, 200X.

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