Product Description

Normal Human Bronchial Epithelial (NHBE) cells exhibit cobblestone morphology in monolayer culture (Figure 1) and are characterized by positive staining for cytokeratin 14 (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. NHBE cells are available from non-diseased donors, donors with a history of smoking, and donors with airway diseases, such as asthma and COPD.

Intended Use

NHBE cells require the use of NHBE Growth Medium (NHBE-GM) for optimal expansion in monolayer culture. When used with NHBE-GM, NHBE cells provide an ideal serum-free culture system to study cellular differentiation and tissue development, cell-matrix interactions, wound healing, toxicity testing and other related research applications.

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

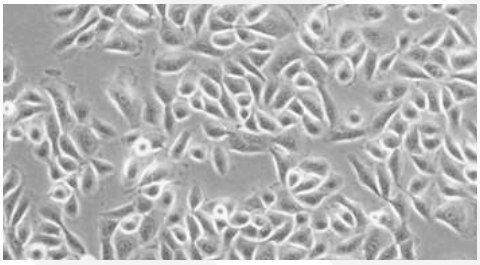

Figure 1. Phase contrast image of NHBE cells in monolayer culture, 100X.

Format

NHBE cells 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 reagent storage conditions.

Directions for Use:

Preparation of NHBE Growth Medium (NHBE-GM)

1. Thaw the growth supplement (NHBE-GS) and hydrocortisone supplement (NHBE-HCS) at room temperature for 30 minutes or until completely thawed. Be sure that all components are in solution and no precipitate is visible. Protect the NHBE-GM from light.
2. Aseptically transfer the contents of one growth supplement vial and one hydrocortisone supplement vial into one 500 mL bottle of basal medium (NHBE-BM).
3. Cap the bottle and swirl to mix.

Note: NHBE-BM supplemented with NHBE-GS and NHBE-HCS is referred to as NHBE-GM. NHBE-GM has an expiration date of one month when stored at 2-8°C. NHBE-GM is antibiotic-free, however antibiotics may be added. If desired, up to 50 µg/mL Gentamycin (Gibco Cat# 15750-060) and 50 ng/mL Fungizone (Gibco Cat# 15920-018) may be used without compromising cell growth or function.

Thaw and Seed Cryopreserved Cells

1. The recommended seeding density for NHBE cells is 3,300 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. When only a sliver of ice remains, remove the vial and wipe with 70% ethanol before opening in a biosafety cabinet.
3. Transfer cells from the cryovial into a sterile conical tube containing 30 mL of NHBE-GM at room temperature. 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.

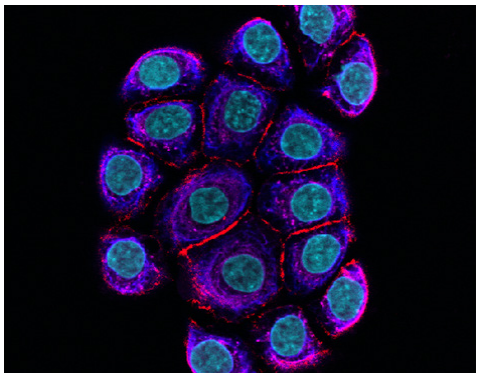

Figure 2. Immunofluorescent characterization of NHBE cells showing cytokeratin 14 (purple), phalloidin (red), and Ki67 (green) expression, 600X.

Maintenance of Monolayer Cell Cultures

1. Change the 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 media. Warm only the required volume of medium as needed.

Subculturing of Cells

1. Subculture the cells when they are 70-80% confluent and contain many mitotic figures (generally 4-5 days after plating).
2. Prepare sufficient Trypsin (0.025%)/EDTA (0.265 mM) solution in Ca^{2+}/Mg^{2+}-free PBS (DPBS) and Soybean Trypsin Inhibitor solution (STI, 0.25 mg/mL) in DPBS at room temperature (0.33 mL/5 cm^2 culture area).
3. Aspirate the medium from the culture vessel and rinse once with DPBS (0.33 mL/5 cm^2 culture area).
4. Add Trypsin/EDTA solution (0.165 mL/5 cm^2 culture area) and gently rock to ensure complete coverage. Incubate at 37°C for 10-15 min or until cells completely round up.
5. Continue trypsinization until ~80% of 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 trypsin exposure to the minimum time necessary.
6. After the cells have been detached, add STI solution (0.33 mL/5 cm^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 combine with the cell suspension.
8. Take a small aliquot for cell counting and centrifuge the remaining suspension at 150 X g for 10 minutes.
9. Resuspend the pellet in fresh, warm NHBE-GM. Seed cells into a new culture vessel at a density of 3,300 cells/cm^2.

Table 1. Reagents for use with NHBE cells

Product	Catalog Number	Size	Storage
Normal Human Bronchial Epithelial Cells (Non-diseased)	NHBE-CRY	≥5x10^5 cells (1 mL)	Liquid Nitrogen Vapor
Normal Human Bronchial Epithelial Cells (Diseased)	NHBE-CRY-DS		
NHBE Growth Medium (NHBE-BM + NHBE-GS + NHBE-HCS)	NHBE-GM		2-8°C
NHBE Basal Medium	NHBE-BM	500 mL	2-8°C
NHBE Growth Supplement	NHBE-GS	10 mL	≤-15°C
NHBE Hydrocortisone Supplement	NHBE-HCS	250 µL	≤-15°C

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