Innovative Life Science Solutions™

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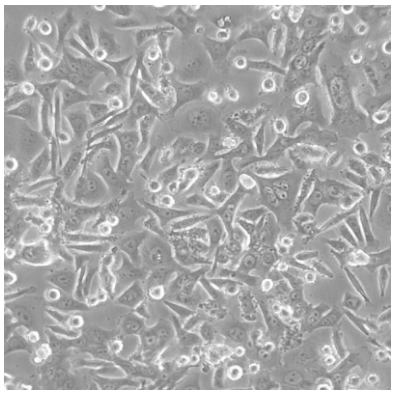
Product Description ©2018

NCM356D™: A NORMAL COLON MUCOSA (NCM) EPITHELIAL-DERIVED CELL LINE

General Description

The NCM356D™ cell line, which was derived originally as NCM356 from normal human colon mucosal epithelium, has proved useful in multiple intestinal research areas including infectious disease, cell signaling, cytokine production, vitamin transport, gene regulation, protein expression, and phosphorylation in multiple growth regulatory pathways. NCM356D™ cells express colonic epithelial cell-associated antigens, such as cytokeratins and villin, but are negative for antigens associated with other cell types, such as neural or endothelial cells. Some of the cells are positive for mucin synthesis as determined by standard staining methods, and the population doubling time is 32 to 35 hours. The NCM356D™ cells are routinely grown in plastic cell culture monolayer flasks as a mixed monolayer/ suspension culture (although the monolayer cells predominate). Initial characterization of the derived NCM356D™ cell line showed that it had normal growth features and was not tumorigenic, but over the long time period it has been in culture, it has acquired some transformation-associated characteristics[1].

Phase Contrast Micrograph NCM356D Cells in Culture



Source

NCM356D™ is an epithelial cell line derived from the normal colon mucosa wide margin resection of a 65-year-old black male with rectal adenocarcinoma and selected for *in vitro* growth (Stauffer et al., 1995). It was not infected or transfected with any exogenous genetic information.

Re-Animation of Cells from Cryovials

Pre-warm the appropriate volume of medium for culture. To re-animate cryostored cells, use safe handling practices and thaw cryovial(s) rapidly (<5 min) in a 37°C water bath. For maximum viability, the whole process should be completed within 30 minutes. Wipe down outside of vial with 70% ethanol prior to opening. Gently and aseptically transfer cells to 2 mL warmed culture medium in a 15 mL conical centrifuge tube. Add about 4 mL of additional growth medium, pellet the cells by centrifugation and add 10 mL fresh complete growth medium to re-suspend cells. Transfer cells to a 75-cm^2 culture flask and incubate at 37°C in 5% CO_2 and air.

Propagation Conditions

Growth media. NCM356D™ cells have fastidious growth requirements and must be maintained in INCELL's enriched M3 media, M3:10™ medium (M310A; which is M3 Base medium plus supplements and 10% [v/v] fetal bovine serum [FBS], and contains antibiotics; OR M310F; which is free of antibiotics) for long-term *in vitro* culture maintenance.

An alternative to purchasing M3:10™ is to supplement M3Base™ with 10% FBS, since M3Base™ contains all the other needed growth supplements. M3Base™ can be purchased with antibiotics (M3:BaseA; Cat #M300A) or without antibiotics (M3:BaseF; Cat #M300F), but if used to culture NCM356D™ cells it must be supplemented with high quality, cell culture tested 10% v/v FBS (antibiotics may be optionally added to M300F medium). The

[1] Initial characterization showed that NCM356 cells did not grow in soft agar and were non-tumorigenic (Stauffer et al, 1995). Recent studies primarily done by INCELL collaborators have demonstrated that the NCM356 cell line and selected subpopulations have variable ability to grow in soft agar, display an abnormal karyotype and some cells have been selected to be tumorigenic. The exact conditions and cell densities used in these assays are inconsistent between research groups. However, our current interpretation is that as a result of in vitro selection, the cell line expresses a transformed phenotype but retains many functional aspects and attributes of normal epithelial colon cells. Individual researchers should assess this information and experimental data for suitability of use in their individual studies or applications.

M3Base™ formulas are recommended for international customers and collaborators because INCELL does not ship FBS-containing media overseas. Media are supplied as 100 mL, 500 mL or as custom packaging (with a 3-4 weeks lead time).

Other growth conditions. Temperature: 37°C; Gas and environment: 95% air, 5% CO_2; humidified.

Subculturing

Do not let the cells overgrow for long periods or the tight clusters of cells in suspension may be difficult to dissociate. The monolayers can be easily subcultured after removal with standard dissociating agents, such as trypsin:EDTA (T:E)[2] or similar solutions. Keep the suspension cells and at least 25% of the spent medium (it contains undefined paracrine factors) upon subculturing.

Procedure. Plan the subculturing split ratio to new flasks or plates, etc. and the volume of spent medium to be retained (i.e., at least 25% of the final media volume). Rock the culture flask gently to release weakly adherent cells into the medium. Aseptically remove all of the spent culture medium (it contains cells in suspension) and transfer to a centrifuge tube. Gently pre-rinse the monolayer with a divalent cation-free buffer such as calcium and magnesium free phosphate buffered saline or with an isotonic saline solution. Save the rinses in another centrifuge tube. Pellet the cells in the tubes by centrifugation at 500 *x g*. Remove and discard 50% to 75% of the spent medium volume from the first tube and all of the saline from the second tube. Triturate the cell pellet in the spent medium, then transfer to the cell pellet from the rinses, then triturate to re-suspend the pooled cells in the spent medium.

Add 2 mL T:E to the cell monolayer in a 75-cm^2 flask and incubate at 37°C for 5-10 min. Tap the flask gently to remove the attached cells. Add complete medium to stop the action of the trypsin. Pool and resuspend all of the cells for counting. Counts can be done electronically (unless there is a lot of clumping) or by using a Hemocytometer with a dye exclusion assay such as Trypan blue (e.g., 0.4% dye; 1-2 minutes then count). Transfer the cells to new culture vessels or plates at split ratios that should not exceed 1:4. Seed at a density of 2 to 8 x 10^5 cells per 10 to 20 mL complete medium volume in a 75-cm^2 flask (or a similar proportional cell number to surface area or volume ratio). The culture will increase in density over a period several days, with a somewhat slow population doubling time of 32-35 hrs.

Cryopreservation

Cells can be cryostored in INCELL's cryopreservation media, including CPZ™ (Cat # MCPZF) or StorZ™ (Cat # STZ) used at 1X concentration; or EZ-CPZ™ (Cat # EZCZ) which is used at 2X concentration and is mixed 1:1 (v:v) with cells suspended in their preferred M3:10™ growth medium or conditioned medium. Use standard methods of slow-freeze (controlled rate freezer or isopropanol containers such as a "Mr. Frosty"), and a rapid thaw (37°C water bath) for re-animation. The storage temperature should be below -130°C, preferably in liquid nitrogen vapor phase.

Biohazard and Infectious Agent Considerations

All human cells should be handled according to International, NIH and CDC guidelines. This cell line was developed from a patient not positive for any known infectious agents, including HIV or hepatitis viruses, B or C. Although the line has not specifically been tested for other human viruses, it is negative for HIV. Bacterial, fungal and mycoplasma tests are negative. SAFETY PRECAUTIONS: Wear gloves and eye protection when thawing cells. Never put vials into liquid nitrogen in the liquid phase as that will compromise the integrity of the vials and they may explode when thawed.

Cell Line Distribution and Publications

INCELL licenses the cell line for use by investigators with a Cell License Material Transfer Agreement (CLMTA). Contact INCELL by email to info@incell.com, FAX 210-877-0200, or phone 800-364-1765 for more details, to request a form, or obtain the NCM publications list, also posted on the www.incell.com website.

[2] 0.05% trypsin:0.02% disodium ethylenediaminetetraacetic acid; final concentrations)