



Innovative Life Science Solutions™

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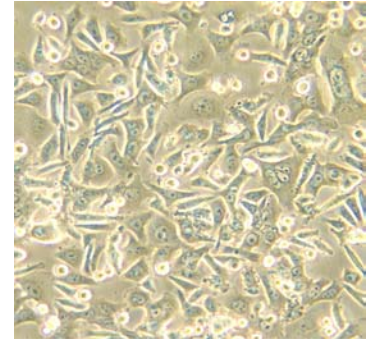
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NORMAL-DERIVED COLON MUCOSA (NCM356)

General Description

The NCM356 cell line, which was derived 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. NCM356 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 32-35 hours. The NCM356 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 NCM356 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 characteristics¹.

**Phase Contrast Micrograph
NCM356 Cells in Culture**



Source

The NCM356 cell line 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. It was not infected or transfected with any exogenous genetic information.

Reconstitution from Cryovials

Thaw cryovial(s) rapidly in a 37°C water bath. For maximum viability, the whole process should be completed within 30 minutes. 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, then add 10 ml fresh, complete growth medium and re-suspend the cells. Transfer the cell suspension to a 75-cm² culture flask and incubate at 37°C in 5% CO₂ and air.

Propagation Conditions

Growth medium: The NCM356 cell line has fastidious growth requirements and must be maintained in INCELL's enriched **M3:10™ medium (Cat # M310A-500;** which is M3 medium plus supplements and 10% [v/v] fetal bovine serum [FBS], and contains antibiotics; OR **M310F-500;** which does not contain antibiotics) for long-term *in vitro* culture maintenance. **M3™ Base (Cat # M300A-500;** contains growth supplements but no antibiotics) may also be used, but it must be supplemented with high quality, cell culture tested 10% FBS (antibiotics may be optionally added to the medium). M3™ Base is recommended for use by international customers, because INCELL does not ship FBS-containing medias overseas. Other growth conditions: 37°C in 95% air, 5% CO₂ humidified environment.

Subculturing

Seed at a density of 2 to 8 x 10⁵ cells per 10-20 ml complete medium in a 75-cm² flask (or a proportional cell number to volume ratio). The culture will increase in density 2- to 10-fold. Monolayers can be subcultured after removal with standard dissociating agents. Split ratios should not exceed 1:4. It is best to maintain the suspension cells upon feeding or subculture. This is done by centrifuging the spent medium (500 x g) to pellet the cells and returning the

¹ Initial characterization showed that cells did not grow in soft agar and were non-tumorigenic. Recent studies primarily done by INCELL collaborators have demonstrated that the NCM356 cell line and another NCM derived cell line NCM460 (1) and their selected cell line subpopulations have variable ability to grow in soft agar, display an abnormal karyotype and may be tumorigenic. The exact conditions, cell densities, and testing protocols used in these assays are inconsistent among 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 of normal epithelial colon cells. Individual researchers should assess this information and experimental data for suitability of use in their individual studies or applications. Although data are more extensive for the NCM460 cell line, we have updated the product specification sheet for the NCM356 cell line to reflect this updated information and have included selected references to use of NCM cell lines.

cells in the pellet to the flask. Re-suspension of the pellet can be done by adding some fresh culture medium or by leaving a residual volume of the spent culture medium (to comprise 25-50% of the total volume of fresh medium).

Cryopreservation

Cells are cryopreserved in INCELL's ready-to-use **CPZ (Cat num: MCPZF)** using standard methods of slow-freeze and rapid thaw for re-animation. Storage temperature: Liquid nitrogen vapor phase.

Biohazard and Infectious Agent Considerations

All human cells should be handled according to NIH and CDC guidelines. This cell line was developed from a patient not known to be positive for 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.

Cell Line Distribution

Cells are distributed by INCELL to investigators either as a collaborative research option established with a Material Transfer Agreement (MTA) or as a Cell Licensing Agreement (CLA). Either form can be requested by email to mtacla@incell.com, or by FAX 210-877-0200.

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