



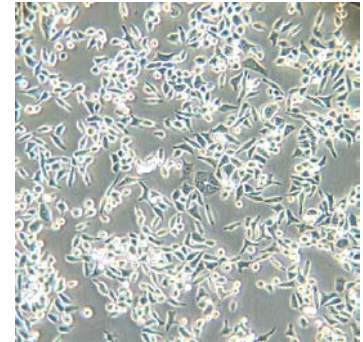
Innovative Life Science Solutions Shaping Global Health™

## NORMAL DERIVED COLON MUCOSA (NCM460)

### General Description

The NCM460 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. NCM460 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 about 32 hours. NCM460 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 NCM460 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<sup>1</sup>.

Phase Contrast Micrograph  
NCM460 Cells in Culture



### Source

The epithelial cell line was derived from the normal colon mucosa of a 68-year old Hispanic male (Moyer et al., 1996) 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-cm<sup>2</sup> culture flask and incubate at 37°C in 5% CO<sub>2</sub> and air.

### Propagation Conditions

Growth medium: NCM460's have fastidious growth requirements and must be maintained in INCELL's enriched **M3:10™ medium (Cat # M310A500)**; which is M3 medium plus supplements and 10% [v/v] fetal bovine serum [FBS], and contains antibiotics; OR **M310F500**; which does not contain antibiotics) for long-term *in vitro* culture maintenance. **M3Base™ (Cat # M300A500)**; contains growth supplements but no antibiotics) may also be used, but it must be supplemented with high quality, cell

<sup>1</sup> Initial characterization showed that NCM460 cells did not grow in soft agar and were non-tumorigenic (Moyer et al, 1996). Recent studies primarily done by INCELL collaborators have demonstrated that the NCM460 cell line and selected subpopulations have variable ability to grow in soft agar, display an abnormal karyotype and may 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 of normal epithelial colon cells. Individual researchers should assess this information and experimental data for suitability of use in their individual studies or applications.

culture tested 10% FBS (antibiotics may be optionally added to the medium). M3Base™ 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% CO<sub>2</sub> humidified environment.

### **Subculturing**

Seed at a density of 2 to 8 x 10<sup>5</sup> cells per 10-20 ml complete medium in a 75-cm<sup>2</sup> 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 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 positive for 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 were negative.

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