Organism: Homo sapiens, human
Disease: Accident victim
Cell Type: Epithelial
Age: 20 years
Gender: female
Morphology: epithelial
Growth Properties: adherent

Unpacking & Storage Instructions

Handling Procedure for Frozen Cells

1. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Flask Cultures

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still...
2. If the cells are still attached, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.

3. If the cells are not attached, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

**Subculturing Procedure**

**Subcultivation Ratio:** A subcultivation ratio of 1:50 to 1:500 is recommended

**Medium Renewal:** Every 2 to 3 days

Remove medium, add dissociation solution (0.02% EDTA and 5% dialized fetal bovine serum in Ca-Mg free Hanks' BSS) and allow the flask to sit at 37°C for 12 minutes or until the cells detach.

Add fresh culture medium, aspirate and dispense into new culture flasks.

Subculture weekly.

**Cryopreservation Medium**

Complete culture medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

**Comments**

NL20 (ATCC CRL-2503) is an immortalized, nontumorigenic human bronchial epithelial cell line derived from normal bronchus taken from an accident victim at autopsy.

The cell line was established by transfection with the origin of replication-defective SV40 large T plasmid, p129.

NL20 cells at passage 183 were inoculated into nude mice and a slowly growing subcutaneous tumor developed from a minor clone in this otherwise stable cell line.

After three months the tumor was removed and placed in culture. At passage 3, these cells were re-injected into nude mice.

One of the resulting tumors was dissociated, placed in culture and designated NL20-TA. This cell line (ATCC CRL-2504) remains tumorigenic up to at least passage 250.

Neoplastic transformation of the NL20 cell line was associated with loss of chromosome 18 together with acquisition of multiple copies of 9q21.2->34.

The non-tumorigenic NL20 cell line and the tumorigenic NL20-TA cell line form a pair of immortal cell lines that can be used to study tumor progression.

**References**

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

**Biosafety Level: 2**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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