





Product Sheet

SW480 [SW-480] (ATCC®) CCL-228™

Please read this **FIRST**



Storage Temp.
liquid nitrogen
vapor phase



Biosafety Level
1

Intended Use

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

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
(Note: The L-15 medium formulation was devised for use in a free gas exchange with atmospheric air. A CO₂ and air mixture is detrimental to cells when using this medium for cultivation)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: SW480 [SW-480] (ATCC® CCL-228™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue: colon
Disease: Dukes' type B, colorectal adenocarcinoma
Age: 50 years
Gender: male
Morphology: epithelial
Growth Properties: adherent
Isoenzymes:
ES-D, 1
G6PD, B
PEP-D, 1
PGD, A
PGM1, 2
PGM3, 1
DNA Profile:
Amelogenin: X
CSF1PO: 13,14
D13S317: 12
D16S539: 13
D5S818: 13
D7S820: 8
THO1: 8
TPOX: 11
vWA: 16

Cytogenetic Analysis: The stemline chromosome number is hypotriploid and 11-12 marker chromosomes were common. Both double minutes and dicentrics were observed in 8% of each metaphase examined.

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. 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 Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a new culture flask.
5. Incubate the culture at 37°C incubator **without CO₂**.



Handling Procedure for Flask Cultures



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The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

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 viable).
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 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 x g 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 in a 37°C incubator **without CO₂**, until cells are ready to be subcultured.



Subculturing Procedure

Volumes are given for a 75 cm² flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C, without CO₂.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:8 is recommended

Medium Renewal: 1 to 2 times per week



Cryopreservation Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.



Comments

A cell line established from a lymph node metastasis taken from the same patient one year later is available (see ATCC CCL-227).

The line is negative for CSAp (CSAp-) and colon antigen 3.

The cells are positive for keratin by immunoperoxidase staining.

There is a G -> A mutation in codon 273 of the p53 gene resulting in an Arg -> His substitution and a C -> T mutation in codon 309 resulting in a Pro -> Ser substitution.

The cells express elevated levels of p53 protein.

The line is positive for expression of c-myc, K-ras, H-ras, N-ras, myb, sis and fos oncogenes.

N-myc oncogene expression was not detected.

Matriysin, a metalloproteinase associated with tumor invasiveness, is not expressed.

The cells have been reported to produce GM-CSF.



References

References and other information relating to this product are available online at www.atcc.org.



Biosafety Level: 1

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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Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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