NCI-H498 [H498]

CCL-254™

Description

Organism: *Homo sapiens*, human
Tissue: Large intestine; Cecum
Age: 56 years
Gender: Male
Morphology: epithelial
Growth properties: Mixed: suspension with some loosely adherent cells
Disease: Adenocarcinoma; Colorectal

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

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

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Unpacking and 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.

Complete medium: The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, ATCC 30-2001. To make the complete growth medium, add the following

components to the base medium: fetal bovine serum (ATCC 30-2020) to a final concentration of 10%.

Handling Procedure:

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.

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 submersed 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.

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 centrifuge cells at 200 x g for 10 minutes. Discard supernatant.

4. Resuspend the cell pellet with the recommended complete medium at the seeding dilution of 1:10 (in a T-25) or 1:15 (in a T-75). Take care not to over pipette. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

5. Incubate the culture at **37°C** in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Special growth requirements:

CCL-254 cells grow as aggregates in suspension. CCL-254 has a history of very low post-thaw viability. Cultures may lose viability when the aggregates are dispersed. Therefore, cell counts are not performed while expanding the CCL-254 cell line.

Please note since the cells cannot be counted, at ATCC approximately 20-mL of viable culture is cryopreserved per ampoule frozen.

Subculturing procedure:
Protocol: Subculture Method

- CCL-254 cultures are maintained by addition of fresh medium.
- A split ratio of 1:3 to 1:4 is used when subculturing CCL-254.
- The CCL-254 cell line is sub cultured when there are numerous, healthy appearing clusters present in suspension.
- When the cell concentration becomes heavy with large clumps, aspirate well and dilute approximately 1:10. If the clumps are allowed to get too large, most of the cells will die.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended
Medium Renewal: Every 2 to 3 days
Reagents for cryopreservation: Complete growth medium supplemented with 10% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: NCI-H498 [H498] (ATCC CCL-254)

References

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

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