ATCC

Product Sheet

CHLA-01-MED (ATCC® CRL-3021™)

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

DMEM:F12 Medium (ATCC 30-2006) with 20 ng/mL human recombinant EGF, 20 ng/mL human recombinant basic FGF, and B-27 Supplement (Invitrogen, Cat. No.17504) to a final concentration of 2% (v/v)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: CHLA-01-MED (ATCC® CRL-3021™)

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: brain/brain tumor
Disease: medulloblastoma
Cell Type: Medulloblastoma
Age: 8 years
Gender: male
Morphology: rounded
Growth Properties: single cells and tight clusters in suspension
DNA Profile:
Amelogenin: X Y
CSF1PO: 11
D13S317: 8, 14
D16S539: 9, 11
D5S818: 11, 13
D7S820: 10
THO1: 6, 9.3
TPOX: 9, 12
vWA: 16, 17
Cytogenetic Analysis: This is a human male cell line with a chromosome range of 42~46. The karyotype contained two consistent chromosome rearrangements, der(17)t(1;17)(q11;q25) and der(18)(17;18)(q11.2;p11.2) and approximately 40% of the examined metaphase spreads contained 11~16 double minutes.

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 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.

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 25 cm^2 or a 75 cm^2 culture flask. 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.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information), grown, and completely filled with medium at

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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
2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium and transfer to culture flask.
3. Incubate the culture, horizontally, at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

Subculturing Procedure

Cultures can be maintained by the addition of fresh medium when there are numerous, healthy-appearing clusters present in suspension and pH of medium has decreased. Alternatively, cultures can be established by centrifugation with subsequent resuspension in ¼ volume of the conditioned medium and ¾ volume fresh medium.
Note: Due to large, tight clusters formed by these cells, it may be difficult to accurately measure cell number and viability.
Subcultivation ratio: A subcultivation ratio of 1:2 to 1:3 is recommended.
Medium renewal: Add fresh medium every 4 to 7 days (depending on cell density).

Cryopreservation Medium

Fetal Bovine Serum (FBS), 50%; Cell Freezing Medium-DMSO (Sigma, Cat. No.C-6295), 50%
ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500 ml) and ATCC® Catalog No. 30-2021 (100ml).

Comments

CHLA-01-MED was established from an 8 year old boy with CNS-disseminated medulloblastoma. Cells were isolated from the brain tumor resected at the time of diagnosis and were cultured in neurobasal medium after mechanical disruption. This cell line has been continuously carried in culture for over 18 months, and in neurobasal medium grows as spheres of varying sizes. When placed in medium containing fetal bovine serum the cells attach and spread, but after a period of proliferation they senesce. The generation of this cell line was made possible with the support of the Michael Hoefflin Foundation for Children's Cancer to Childrens Hospital Los Angeles, with the goal of making pediatric brain tumor lines available to the research community. A metastatic recurrent medulloblastoma cell line from this patient is available as CHLA-01R-MED (see ATCC CRL-3034)

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.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet,

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