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

Iscove's Modified Dulbecco's Medium (IMDM; ATCC® No. 30-2005); RPMI-1640 Medium (ATCC® No. 30-2001) (4:1) + 10% FBS (ATCC® No. 30-2020) + additional 1% L-glutamine (ATCC® No. 30-2214)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: U-CH2 (ATCC® CRL-3218™)

Shipping Information

frozen

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 25 cm² or a 75 cm² collagen-coated-culture flask (see subculture on product web page for the coating procedure). 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% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

Temperature: 37°C
Atmosphere: air, 95%; carbon dioxide (CO₂), 5%

Subculturing Procedure
Coating description: Dilute rat tail type I collagen (BD Biosciences, Catalog No. 354236) to 50 μg/ml. Add 7.5 ml coating buffer to flask and incubate at room temperature for one hour. Carefully aspirate remaining solution. Rinse flask 2 times to remove acid, using 1x DPBS. Coated flasks may be used immediately or stored at 2-8°C up to one week under sterile conditions.

Volumes used in this subculture protocol are for a 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 5.0 ml Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 5.0 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 5.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
6. Resuspend the cell pellet in 10 ml fresh growth medium.
7. Add appropriate aliquots of the cell suspension to new coated culture vessels.
8. Incubate cultures at 37°C.

**Cryopreservation Medium**

Freeze medium: 70% complete growth medium supplemented with an additional 20% fetal bovine serum and 10% DMSO

**Comments**

U-CH2 is a human chordoma cell line that was established from tumor tissue obtained from a 72-year old female patient with recurrent sacral chordoma. It exhibits chordoma-like characteristics, and has molecular, genetic, and morphological features typical of chordoma. Chordoma is a rare slow-growing tumor type, and U-CH2 is a relatively slow-growing cell line. U-CH2 has a heterogeneous morphology consisting of physaliferous cells with mucinous intercellular substance, which represent typical chordoma features. The cells contain amplification of transcription factor T (Brachyury) that is most specific marker for chordoma. This cell line was accessioned with the support of the Chordoma Foundation, a nonprofit organization working to improve the lives of chordoma patients by accelerating research to develop effective treatments for chordoma.

**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**

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

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