ATCC

Product Sheet

CTLL-2 (ATCC® TIB-214™)

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 RPMI-1640 Medium, (ATCC® 30-2001™). To make the complete growth medium, add the following components to the base medium: additional 2 mM L-glutamine; additional 1mM sodium pyruvate; adjust to a final concentration of 10% fetal bovine serum and 10% T-STIM with Con A. T-STIM is available from Becton Dickinson.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: CTLL-2 (ATCC® TIB-214™)

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: *Mus musculus*, mouse
Strain: C57BL/6
Cell Type: lymphocyte cytotoxic T lymphocyte;
Morphology: lymphoblast
Growth Properties: suspension

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. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium. Adjust the cell density of the suspension to 1 X 10^5 viable cells/mL.
4. Transfer cells to an appropriate size vessel. 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 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.

Handling Procedure for Flask Cultures

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
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.
3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 2-5 X 10^4 viable cells/ml in the shipping medium.

Subculturing Procedure

Subculture actively growing suspension cultures before they have reached 2 X 10^5 cells/ml or the IL-2 will

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rapidly deplete and the cells will quickly lose viability Use inoculation densities of 1 to 2 X 10^4 viable cells/mL. Corning® T-75 flasks (catalog #431464) are recommended for subculturing this product.
Medium Renewal: Twice per week
Some Important Considerations in Handling CTLL-2, TIB-214
Frozen Cells: Viability immediately after thawing will be 70-80%. Expect viability to be very poor from day 1 to day 4 after culture initiation. Culture will appear to be completely dead. On the third to fifth day following initiation viable cell clusters will begin to appear in suspension. Usually cells will be ready to subculture on the 7th to the 10th day after the ampule is thawed. However, it may take from 2-3 weeks before vigorous growth is observed. It is best to leave the initial culture undisturbed until cells enter their growth phase.
Overgrowth: In the event cell density becomes too great and viability decreases to where culture appears totally dead, the culture may still be rescued. Inoculate a flask at a density of 1 X 10^4 viable cells/mL.

Cryopreservation Medium

Complete growth medium described above supplemented with an additional 10% FBS and 7.5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

The cells are dependent upon IL-2 for growth, and can be used to assay for IL-2. Immediately after thawing (or after overgrowth of a culture) the culture will appear to have few or no viable cells; this is normal. Depending upon the source and potency of the IL-2, it may take up to three weeks before the cells are ready to subculture. T-STIM with Con A (rat IL-2 culture supplement from Becton Dickinson) may be used or the rat factor may be prepared as described below. The freshly prepared rat factor will usually produce more rapid growth. Tested and found negative for ectromelia virus (mousepox).
T-STIM with Con A (rat IL-2 culture supplement from Becton Dickinson) may be used or the rat factor may be prepared as described on the product sheet. The freshly prepared rat factor will usually produce more rapid growth.
Rat Growth Factor (Laboratory Preparation)
Spleens are removed from female Sprague-Dawley rats weighing 200 g. They are coarsely minced before passing through a No. 60 sieve. Wash cells 2-3 times with RPMI 1640. Resuspend cells at 1-1.5 X 10^6 viable cells/ml with 100-200 mL/150 cm^2 flask in RPMI 1640 containing 1% heat-inactivated fetal bovine serum, 0.05 mM, 2-mercaptoethanol, 15 mM HEPES, 100 units/mL penicillin, 100 mg/mL streptomycin and 1.0 µg/mL concanavalin A.
Incubate at 37°C in a CO2 incubator for 48 hours. Harvest the supernatant by centrifugation at 16,000 x g for 10 minutes at 4°C. Sterilize by filtration using a 0.22 micron membrane.
Store at -60°C. Avoid freeze-thaw cycles. Rat growth factor stored at 4°C up to one month has retained its quality. The growth factor is added to the medium just before use. Expect 1-2 X 10^8 cells per spleen which yields 100-200 mL of growth factor.

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.

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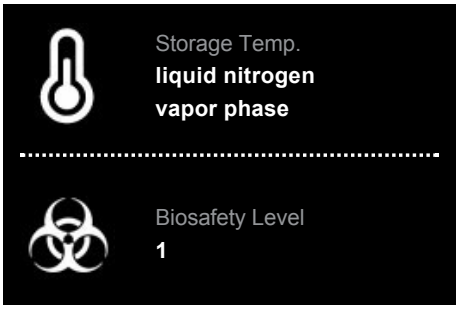

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