LUHMES

CRL-2927™

Description

LUHMES is a neuronal-like cell that was isolated from the mesencephalon of a patient. This cell line was deposited by J Lotharius in 1998 and can be used in neuroscience research.

Organism: *Homo sapiens*, human
Tissue: Brain; Mesencephalon
Age: 8 weeks gestation
Morphology: neuronal
Growth properties: Adherent

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 2

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.

Cells contain v-myc retroviral vector

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 DMEM:F12 Medium Catalog No. 30-2006. To make the complete growth medium, add the following components to the base medium:

- 1% N2 supplement (Gibco-Invitrogen Cat No 17502-048)
- 40 ng/ml b-FGF (basic recombinant human Fibroblast Growth Factor; Gibco-Invitrogen Cat No 13256-029) **added fresh at the last moment**

Handling Procedure:

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.

Note: Culture flasks should be **pre-coated** with 50µg/mL poly-L-ornithine and then with 1µg/mL human fibronectin. (See Subculturing Procedure)

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 culture medium without bFGF and spin at approximately 125 x g for 5 to 10 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 **pre-coated** vented 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.

Subculturing procedure:

These cells should be recovered from cryopreservation and subcultured only on

culture flasks that are sequentially **pre-coated** with 50 µg/mL poly-L-ornithine (Sigma, Cat. No. P-3655 or equivalent) and then with 1 µg/mL Human Fibronectin (Sigma, Cat. No. F-0895 or equivalent).

Note: Use flasks with vented caps for best results.

1. Add 7.0 mL freshly diluted 50 µg/mL poly-L-ornithine to T-75 cm^2 flask and allow flask to sit over night at room temperature.
2. Remove and discard poly-L-ornithine solution. Rinse flask 3 times with culture grade water (Hyclone cat#SH30529.01). Discard last rinse and allow flask to air-dry uncapped and standing upright in a biological cabinet.
3. Add 5.0 mL freshly diluted 1 µg/mL fibronectin and incubate 3 hours at 37°C.
4. Remove and discard fibronectin solution. Rinse flask 3 times with culture grade water (Hyclone cat#SH30529.01). Discard last rinse and allow flask to air-dry uncapped and standing upright in a biological cabinet.
5. Flask is ready for use when dry.

Volumes used in this protocol are for 75 cm^2 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: Subculture before cells reach 80% confluency. Warm aliquots of wash medium (growth medium without bFGF) used in Step 4, freshly made complete growth medium and freshly diluted tryspin solution to 37°C prior to use.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 10.0 mL Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS).
3. Add 4.0 mL of freshly diluted, pre-warmed 0.025% Trypsin-0.1 g/L EDTA solution (see formula below) to flask and incubate for 3 minutes at 37°C. Knock the flask several times against the palm of your hand to dislodge cells. Observe flask under an inverted microscope to be sure cells have come off.
4. Add 6.0 mL of pre-warmed wash medium (see Note above) and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 190 x g for 7 minutes. Discard supernatant and knock the tube against the palm of your hand to loosen the cell pellet. Using a 1 mL pipet, add 1.0 mL complete growth medium and pipet the pellet up and down to resuspend the cells (avoid creating foam or bubbles).
6. Add an additional 2.0 mL of complete growth medium and dissociate cells

further by pipetting up and down.
7. Adjust cell concentration by adding the necessary volume of complete growth medium needed to seed new flasks. Pipet up and down to evenly resuspend cells.
8. Add appropriate aliquots of the cell suspension to new pre-coated vented culture flasks.
9. Incubate cultures in 5% CO_2/95% air at 37°C.

Subcultivation ratio: A subcultivation ratio of 1:3 to 1:4 is recommended. Subculture approximately every 3 to 4 days.
Medium renewal: Every 2 to 3 days

2X Trypsin-EDTA Solution: 2X Trypsin-EDTA solution is 0.05% Trypsin-0.2g/L EDTA. Before use this must be diluted 1:1 in Ca++/Mg++ free Dulbucco's phosphate-buffered saline (D-PBS) to 0.025% Trypsin-0.1g/l EDTA
To make 1 liter of 2X Trypsin-EDTA solution:

1. Add the following to 500 ml ddH_2O:
    a. 8 g NaCl
    b. 0.4 g KCL
    c. 0.58 g $NaHCO_3$
    d. 1 g Dextrose
    e. 0.2 g EDTA
    f. 0.5 g Trypsin (Sigma, Cat. No. T7409)
2. Bring volume up to 1 liter with ddH_2O, and pH to 7.4 with HCL.
3. Incubate at 37°C for at least 1 hour to activate the trypsin.
4. Sterile filter (0.2 µm) and make aliquots.
5. Refrigerate at 4°C overnight and then store at -20°C.
6. Before use, dilute trypsin 1:1 with Ca++/Mg++ free Dulbucco?s phosphate-buffered saline (D-PBS) and warm to 37°C.

Reagents for cryopreservation: Complete growth medium supplemented with 20% fetal bovine serum and 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: LUHMES (ATCC CRL-2927)

References

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

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Contact Information

ATCC
10801 University Boulevard

Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor