ATCC

Product Sheet

LUHMES (ATCC® CRL-2927™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
2

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 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**

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: LUHMES (ATCC® CRL-2927™)

Description

Organism: *Homo sapiens*, human
Tissue: mesencephalon
Age: fetus, 8 weeks gestation
Morphology: neuronal
Growth Properties: adherent
DNA Profile:
Amelogenin: X
CSF1PO: 13,14
D5S818: 11,13
D13S317: 9,11
D7S820: 11,13
D16S539: 11,12
vWA: 14,17
THO1: 7,9.3
TPOX: 8

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.
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.

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.

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Note: Culture flasks should be sequentially **pre-coated** with 50 µg/ml poly-L-ornithine (Sigma, Cat. No. P-3655 or equivalent) and then with 1µg/ml Human Fibronectin (Sigma, Cat. No. F-0895 or equivalent).

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. Also, check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to a **pre-coated** 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

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 sterile double distilled water. 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 sterile water. 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

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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.

Cryopreservation Medium

Complete growth medium, 70%; heat-inactivated FBS, 20%; DMSO, 10%.
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Comments

LUHMES cells can be differentiated into morphologically and biochemically mature dopamine-like neurons following exposure to tetracycline, GDNF (glial cell line-derived neurotrophic factor), and db-cAMP. LUHMES cells exhibit the same dopaminergic and neuronal characteristics as MESC2.10 cells.

References

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

Biosafety Level: 2

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

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. 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 strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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