It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the pellet in 10 mL of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.
Subculturing Procedure

Volumes are given for a 75 cm² flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 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).
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subculture Ratio: A subculture ratio of 1:5 to 1:10 is recommended

Medium Renewal: Twice per week

Cryopreservation Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

The cells express the mRNA for large T antigen.
Lung surfactant proteins B and C were detected.
The cells secrete phospholipids in response to phorbol esters and ATP but not in response to forskolin.

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.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

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

MLE 12 (ATCC® CRL-2110™)

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

HITES medium supplemented with 2% fetal bovine serum

HITES medium with 2% fetal bovine serum is formulated at the ATCC as follows:
- Dulbecco's medium : Ham's F12, 50:50 mix
  (ATCC® 30-2006)
- Insulin 0.005 mg/ml
- Transferrin 0.01 mg/ml
- Sodium selenite 30 nM
- Hydrocortisone 10 nM
- β-estradiol 10 nM
- HEPES 10 mM
- L-glutamine (ATCC® 30-2214) 2 mM (in addition to that in the base medium)
- Fetal bovine serum (ATCC® 30-2020) 2%

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: MLE 12 (ATCC® CRL-2110™)