It is important to note that some vials leak when submersed in liquid nitrogen. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin 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 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.

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% CO₂ 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 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

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.
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. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (v/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which 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).
   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 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.

Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:8 is recommended

Medium Renewal: Every 2 to 3 days

Cryopreservation Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Comments

The MC3T3-E1 Subclone 4 (ATCC CRL-2593) and the MC3T3 Subclone 14 (ATCC CRL-2594) lines exhibit high levels of osteoblast differentiation after growth in ascorbic acid and 3 to 4 mM inorganic phosphate. They form a well mineralized extracellular matrix (ECM) after 10 days. ref

The MC3T3 Subclone 24 (ATCC CRL-2595) and the MC3T3 Subclone 30 (ATCC CRL-2596) lines exhibit poor osteoblast differentiation after growth in ascorbic acid. They do not form ECM. They can be used as negative controls for Subclones 4 and 14. ref

Mineralizing subclones selectively express mRNAs for the osteoblast markers, bone sialoprotein (BSP), osteocalcin (OCN), and the parathyroid hormone (PTH)/parathyroid hormone-related protein (PTHrP) receptor. Subclones with both high and low differentiation potential produce similar amounts of collagen in culture and express comparable basal levels of mRNA encoding Osf2/Cbfa1, an osteoblast-related transcription factor. ref

After implantation into immunodeficient mice, highly differentiating subclones form bone-like ossicles resembling woven bone, while poorly differentiating cells only produce fibrous tissue. ref

These cell lines are good models for studying in vitro osteoblast differentiation, particularly ECM signaling. They have behavior similar to primary calvarial osteoblasts.

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.

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

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