HMC3 (ATCC® CRL-3304™)

Please read this FIRST

Storage Temp.
liquid nitrogen
vapor phase

Biosafety Level
2

Description

Organism: Homo sapiens, human
Tissue: brain
Cell Type: microglia
Age: embryo
Morphology: macrophage
Growth Properties: adherent
DNA Profile:
Amelogenin: X
CSF1PO: 10,11
D13S317: 11
D16S539: 12,13
D5S818: 11,12
D7S820: 9,11
THO1: 6
TPOX: 8,9
vWA: 17,19

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 EMEM (ATCC® 30-2020™). To make the complete medium add 56 mL FBS (ATCC® 30-2020™) to a 500 mL bottle of the base medium.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: HMC3 (ATCC® CRL-3304™)

Introducing the product

Handling Procedure for Frozen Cells

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 Flask Cultures

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

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

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 complete culture medium, and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 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). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO2 in air atmosphere is recommended if using the medium described on this product sheet.
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 pellet 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.

**Subculturing Procedure**

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium 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% (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).
   - 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.
   - Cultures can be established between 1 x 10⁴ and 4 x 10⁴ viable cells/cm². Do not exceed 7 x 10⁴ cells/cm².
6. Incubate cultures at 37°C.

**Interval:** Maintain cultures at a cell concentration between 1.0 x 10⁴ and 2.0 x 10⁵ cell/cm².

**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended.

**Medium Renewal:** 2 to 3 times per week

**Cryopreservation Medium**

- 90% Culture Medium + 10% DMSO

**Comments**

Resting HMC3 cells were strongly positive for the microglia/macrophage marker IBA1, positive for the endotoxin receptor CD14, but negative for the astrocyte marker GFAP. Markers of activated microglia, namely MHCI, CD68 and CD11b were negative in resting HMC3 cells, but upregulated after activation by IFN-gamma (10 ng/ml, 24 h).

**References**

References and other information relating to this product are available online at [www.atcc.org](http://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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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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