Please read this FIRST

Biosafety Level

1

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 Hybri-Care Medium, Catalog No. 46-X. Hybri-Care Medium is supplied as a powder and should be reconstituted in 1 L cell culture grade water. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%
- 1.5 g/L sodium bicarbonate for use with 5% CO₂ in air atmosphere.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: D1-4G2-4-15 (ATCC® HB-112™)

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.

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 two 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 growth medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² 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% CO₂ 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.
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.
2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium.
3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 2 to 3 x 10⁵ viable cells/mL in the shipping medium.
4. Incubate the culture, horizontally, at 37°C in a 5% CO₂ in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

Subculturing Procedure

Medium Renewal: Every 2 to 3 days
Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 2 × 10^5 cells/ml and maintain between 1 × 10^5 and 1 × 10^6 cells/ml.

**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**

Animals were immunized with dengue virus type 2 antigens. The antibody reacts with flavivirus group specific antigens. Tested and found negative for ectromelia virus (mousepox).

**References**

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

**ATCC Warranty**

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

**Disclaimers**

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