ATCC

Product Sheet

H36.12j [Pixie 12j] (ATCC® CRL-2449™)

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-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium:

- heat-inactivated iron supplemented bovine calf serum to a final concentration of 10%

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: H36.12j [Pixie 12j] (ATCC® CRL-2449™)

Description

Organism: *Mus musculus* (macrophage tumor cell line); *Mus musculus* (peritoneal macrophage), mouse(macrophage tumor cell line); mouse (peritoneal macrophage)
Cell Type: Macrophage
Morphology: macrophage
Growth Properties: suspension (some adherent cells)

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

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.
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.
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 xg for 5 to7 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% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

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 xg for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 ml of this medium and return the cells to

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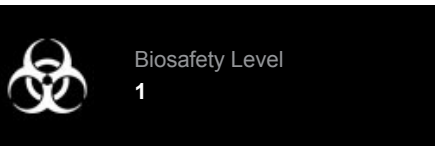

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the shipping flask

3. Incubate the culture, horizontally, at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

Subculturing Procedure

Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density)
Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 to 2 X 10 exp5 viable cells/ml. Maintain cell density between 1 X 10 exp5 and 1 X 10 exp6 viable cells/ml.
Adherent cells may be harvested by scraping.

Cryopreservation Medium

Cryoprotectant 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

This cell line was established by P.A. Campbell and E.P. Canono in 1991. Percoll gradient purified, proteose peptone elicited peritoneal macrophage cells from C57BL/6N mice were fused with drug selected P388D1 mouse macrophage tumor cells.
H36.12j is a model for the permissive intracellular growth of Listeria monocytogenes within mouse macrophages. The cells phagocytose Listeria via receptors for internalin A (InlA), a Listeria surface polypeptide.
The cells produce tumor necrosis factor alpha (TNF alpha) upon direct beryllium stimulation and serve as an in vitro model for chronic beryllium disease in humans.
Since intracellular Listeria escape into the cytoplasm where they replicate, the cell line is a useful tool in the study of the permissive intracellular growth cycle of Listeria.
The H36.12j cells have been found to express biologically active surface IL-10 that regulates macrophage bacterial activity.
These cells may be used in assays for the phagocytosis and killing of microorganisms, in assays for the production of cytokines by mouse macrophages, and for assays for the effect of environmental toxins on mouse macrophages.

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.

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

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC

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