



Product Sheet

10-1.D.2 (ATCC® TIB-165™)

Please read this FIRST



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: 10-1.D.2 (ATCC® TIB-165™)

Description

Organism: *Mus musculus* (B cell); *Mus musculus* (myeloma), mouse (B cell); mouse (myeloma)

Isotype: IgG2b

Cell Type: hybridoma: B lymphocyte

Morphology: lymphoblast

Growth Properties: suspension

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

- Initiate culture as soon as possible upon receipt.

- Thaw by rapid agitation in 37°C water bath. Thawing should be rapid (within 40-60 seconds). As soon as the ice is melted, remove the ampule from the water bath and immerse in 70% ethanol at room temperature. All of the operations from this point on should be carried out under strict aseptic conditions.

- The cells are supplied in two different types of glass ampules. One is a standard ampule, the neck of which must be scored with a sharp file that has been immersed in ethanol. A definitive sharp nick about 1/8" in length on one side is necessary. The second type is prescored and is identifiable by a gold band around the ampule neck, and should not be scored with a file.

- Break the neck of the ampule between several folds of a sterile towel.

- Transfer the cell suspension and dilute it with the recommended culture medium in a culture flask (see specific batch information above for dilution ratio); incubate at 37°C with 10% CO₂ in air atmosphere. Since it is important to avoid excessive alkalinity of the medium during recovery of the cells, it is suggested that the culture medium be placed into the culture flask, tube, etc. and the pH be adjusted, as necessary, prior to the addition of the ampule contents. Note that the bicarbonate content of the culture medium will determine whether an atmosphere containing CO₂ will be required.

- It is not necessary to remove the freezing additive. However, if desired, the culture medium may be changed to remove the protective freezing additive (dimethylsulfoxide) 24 hours after thawing. If it is desired that the freezing additive be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid and resuspend the cells with growth medium at the dilution ratio given in the specific batch information above.

FLUID RENEWAL

Add fresh medium (depending on cell density) every 2-3 days.



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SUBCULTURE PROCEDURE

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 0.5-1 x 10⁵ viable cells/ml. Maintain culture density between 10⁵ and 10⁶ viable cells/ml.



Handling Procedure for Flask Cultures

HANDLING PROCEDURE FOR FLASK CULTURES (SUSPENSION)

The flask was seeded with cells (see specific batch information above for concentration), grown and completely filled with medium to prevent loss of cells in transit. Upon receipt incubate the flask in an upright position for several hours to return the flask contents to 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 300 x g for 15 minutes. Resuspend the cell pellet in 10-12 ml of the shipping medium. From this suspension remove a sample for a cell count and viability so that the cell density of the suspension can be adjusted to 2-3 x 10⁵ viable cells/ml. If the suspension needs to be diluted use the shipping medium. Incubate the culture in a flat position at 37°C. The shipping medium contains reduced sodium bicarbonate suitable for a 5% CO₂ in air incubator. DMEM usually contains 3.7 grams of sodium bicarbonate per liter and should be incubated in a 10% CO₂ in air incubator. Maintain the cell density of the culture as suggested under the subculture procedure described above.

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.
3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 2-3 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 1 x 10⁵ cells/ml maintain between 1 x 10⁵ and 1 x 10⁶ cells/ml.



Cryopreservation Medium

Cryoprotectant 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

Animals were immunized with spleen cells from DBA/2 mice. Spleen cells were fused with Sp2/0-Ag14 myeloma cells. Lyb-2.1 antigen is a mouse B cell differentiation antigen (45000 dalton glycoprotein). The antibody induces blastogenesis in B cells, and this response is limited to the Lyb 5+ class of B cells. B cells activated in this way mature into immunoglobulin secreting cells in the presence of T cell replacing factors (medium from Con A treated T cells).



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Tested and found negative for ectromelia virus (mousepox).



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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