



Product Sheet

## BNL CL.2 (ATCC® TIB-73™)

Please read this FIRST



Storage Temp.  
**liquid nitrogen**  
vapor phase

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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: fetal bovine 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: BNL CL.2 (ATCC® TIB-73™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

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### Description

**Organism:** *Mus musculus*, mouse  
**Strain:** BALB/c  
**Tissue:** liver  
**Disease:** normal  
**Age:** embryo  
**Morphology:** epithelial  
**Growth Properties:** adherent

### 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  $-130^{\circ}\text{C}$ , preferably in liquid nitrogen vapor, until ready for use.

### 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  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

1. Thaw by rapid agitation in  $37^{\circ}\text{C}$  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.
2. 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  $37^{\circ}\text{C}$  with 10%  $\text{CO}_2$  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  $\text{CO}_2$  will be required.
3. 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  $125 \times g$  for 10 minutes, discard the fluid and resuspend the cells with growth medium at the dilution ratio given in the specific batch information.

### Handling Procedure for Flask Cultures

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.

1. Remove all of the medium (which can be saved and used as fresh medium) except for a sufficient volume (5-10 mL) to cover the floor of the flask. The shipping medium contains reduced sodium bicarbonate suitable for a 5%  $\text{CO}_2$  in air incubator. DMEM usually contains 3.7 grams of sodium bicarbonate per liter and should be incubated in a 10%  $\text{CO}_2$  in air incubator.
2. Sometimes in transit the cultures are handled roughly and most of the cells become detached and float in the culture medium. If this has occurred remove the entire contents of the flask and centrifuge at  $300 \times g$  for 15 minutes.
3. Draw off the excess supernatant medium, resuspend the cells in 10 mL of the culture medium and plant the entire cell suspension in a single flask of suitable size (about  $25 \text{ cm}^2$ ).



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## Subculturing Procedure

Volumes used in this protocol are for 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM 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:** 1:2 to 1:6

**Medium Renewal:** Every 2 to 3 days

**Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in *Culture of Animal Cells, a Manual of Basic Technique* by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.



## Cryopreservation Medium

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



## Comments

The line was selected to grow in medium containing ornithine and phenylalanine in place of arginine and tyrosine.

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).



## 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

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## Disclaimers

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product. The MTA is also available on our Web site at [www.atcc.org](http://www.atcc.org)

Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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