




Product Sheet


# Hybridoma, hCD40L-M91 [M91, hCD40L-M91] (ATCC® HB-12056™)

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: Hybridoma, hCD40L-M91 [M91, hCD40L-M91] (ATCC® HB-12056™)

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  
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Or contact your local distributor

## Description

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

**Strain:**

**Strain:** BALB/c (B cell); BALB/c (myeloma)

**Isotype:** mouse IgG1, IgG1

**Cell Type:** hybridoma:lymphoblast 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

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.

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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 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm<sup>2</sup> or a 75 cm<sup>2</sup> 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% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product.

## 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.




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Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium.

- From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to  $2-3 \times 10^5$  viable cells/mL in the shipping medium.
- Incubate the culture, horizontally, at 37°C in a 5% CO<sub>2</sub> in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.



### Subculturing Procedure

**Protocol:** 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  $2 \times 10^5$  viable cells/ml. Maintain cell density between  $1 \times 10^5$  and  $1 \times 10^6$  viable cells/ml.

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



### Cryopreservation Medium

#### Recommended Cryoprotectant Medium

Complete growth medium described above supplemented with 7.5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.



### Comments

Animals were immunized with human CD40 ligand (CD154, CD40L).

Spleen cells were fused with either Ag8.653 or NS1 mouse myeloma cells (the specific line used was not specified).

The antibody recognizes the human CD40L polypeptide.



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

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