



## Product Sheet

# BALB/3T3 clone A31 (ATCC® CCL-163™)

### 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: 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: BALB/3T3 clone A31 (ATCC® CCL-163™)

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

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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:** embryo

**Cell Type:** fibroblast

**Age:** embryo; 14 to 17 days gestation

**Morphology:** fibroblast

**Growth Properties:** adherent

**Cytogenetic Analysis:** modal number = 78; range = 62 to 109. The stemline number is hypotetraploid. Most of the cells contained only telocentric or acrocentric chromosomes. Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

## 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 the vial by gentle agitation in a  $37^{\circ}\text{C}$  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  $125 \times g$  for 5 to 7 minutes. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete medium and dispense into a culture flask (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).
5. Incubate the culture at  $37^{\circ}\text{C}$  in a suitable incubator. A 5%  $\text{CO}_2$  in air atmosphere is recommended if using the medium described on this product sheet.

## 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. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at  $125 \times g$  for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10



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mL of this medium and add to 25 cm<sup>2</sup> flask. Incubate at 37°C in a 5% CO<sub>2</sub> in air atmosphere until cells are ready to be subcultured.



### Subculturing Procedure

Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

**Note:** Never allow cultures to become completely confluent before subculture.

Some important considerations in the handling of 3T3 cells: doubling time is about 18 hours in sparse cultures. The cells reach a saturation density of about 10<sup>6</sup> cells per 20 cm<sup>2</sup>. In order to maintain this property of high contact inhibition, it is necessary to transfer routinely at only high dilutions, otherwise variants tend to be selected having reduced contact inhibition. Such low density makes culture vessels appear sparse and cell growth sensitive to sub-optimal temperature and media conditions.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that 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:** For 60 mm plates, use an inoculum of 3 x 10<sup>5</sup> cells per plate and subculture every 3 days.

**Medium Renewal:** Twice per week

**Note:** The serum used is calf serum, NOT fetal calf serum. The depositor recommended calf serum because fetal calf serum causes transformation and loss of contact inhibition.



### 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 cells are extremely sensitive to contact inhibition of cell division, grow at a high dilution, exhibit a low saturation density and are highly susceptible to transformation in tissue culture by the oncogenic DNA virus, SV40, and murine sarcoma virus

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

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