Product Sheet
C2C12 (ATCC® CRL-1772™)

Please read this FIRST

Storage Temp.
liquid nitrogen
vapor phase

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: C2C12 (ATCC® CRL-1772™)

SAFETY PRECAUTION

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. 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 Flask Cultures

Handling Procedure for Frozen Cells

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. 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 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. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.
5. Transfer the cell suspension to an appropriate size vessel. 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 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).
6. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

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Unpacking & Storage Instructions

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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°C in a 5% CO₂ 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 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

**Subculturing Procedure**

**IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT.**
Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture. Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.

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 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. Inoculate at a cell concentration between 1.5 X 10⁶ and 1.0 X 10⁶ viable cells/75 cm². Coming® T-75 flasks (catalog #430641) are recommended for subculturing this product.

**Medium Renewal:** Every two to three days

**Comments**

The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. Treatment with bone morphogenetic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. Test ed 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.

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