C2C12

CRL-1772™

Description

C2C12 is a myoblast cell line that is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic.

Organism: *Mus musculus*, mouse
Cell Type: myoblast
Tissue: Muscle
Morphology: myoblast
Growth properties: Adherent

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories*

(BMBL), U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Seeding density: 5.0 x 10^3 viable cells/cm^2

Handling Procedures

Unpacking and 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.

Complete medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium (ATCC 30-2002). To make the complete growth medium, add the following components to the base medium: fetal bovine serum (ATCC 30-2020) to a final concentration of 10%.

Handling Procedure:

Handling Procedure for Frozen Cells

To ensure 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. 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 150 to 400 x g for 8 to 12 minutes. (280 x *g* for 10 minutes).
4. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.
5. Resuspend cell pellet with the recommended complete growth medium (see the lot information on Certificate of Analysis (COA) for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask as recommended on the COA. The recommended seeding density for 4.0 x

10^3 to 6.0 x 10^3 viable cells/cm^2. 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% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

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.

Subculture when cells are ≤ 80% confluent.

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 D-PBS (ATCC 30-2200) to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution (ATCC 30-2101) 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. Subculture at a seeding density of 9.0 x 10^3 to 2.0 x 10^4 viable cells/cm^2. Corning T-75 flasks (catalog #430641) are recommended for subculturing this product.
6. Incubate cultures at 37°C.

Medium Renewal: Every two to three days

ATCC Differentiation protocol

Note: Myotube formation is observed in ≤ 14 days

Note: Cells will not grow after differentiation

The differentiation potential is dependent on how the cells have been cultured and subcultured. To prevent the loss of myoblastic cells during regular passaging it is critical that the cells are not allowed to become confluent. The myoblast population of the CRL-1772 cell line will become depleted rapidly if the cultures are allowed to become confluent; this can significantly delay the differentiation of these cells.

During our QC analysis ATCC tests every lot of C2C12 (ATCC CRL-1772) for myotube formation by allowing the cells to become confluent.

At ATCC the CRL-1772 cells are tested for myotube formation as follows:

Seed cells at 6.0 x 10^3 viable cells/cm^2. Observe the culture every workday. The culture should be 80-90% confluent after approximately 3 days with no myotube formation. Continue to observe every 2 to 4 workdays: fluid change as necessary to keep the culture alive. Observe the culture on day 14 for myotube formation.

These cells will differentiate at confluence alone however changing to horse serum after reaching 100% confluency encourages faster myogenesis by reducing the number of growth factors available to the cells. Myotube formation is enhanced when the medium is supplemented with horse serum instead of FBS.

Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: C2C12 (ATCC CRL-1772)

References

References and other information relating to this material are available at www.atcc.org.

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