Cell Line Designation: C2C12

AddexBio Catalog No. P0028001

Cell Line Description:

Disease: Normal

Origin: Derived from normal mouse leg muscle

Species: Mus musculus, C3H, mouse

Tissue: Muscle

Properties: Adherent, Myoblast

Complete Medium: AddexBio-formulated DMEM (C0003-01) + 10% FBS

Subculture Procedure: Seed the cells at a density of less than 5 x 10^3 cells/cm^2. Split sub-confluent culture 1:4 to 1:6 every when 50-70% confluent using trypsin/EDTA; with CO_2; 37°C

Medium Renewal: Two to three times weekly.

Freezing Medium: Complete culture medium supplemented with 5% (v/v) DMSO

Additional Information: Additional product and technical information can be obtained from the catalog references and the Addexbio Technical Information site at www.addexbio.com, or by email at customersupport@addexbio.com.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafty/bmbl4/bmbl4toc.htm

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Use Restrictions: **These cells are distributed for research purposes only.** Addexbio does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Cells Upon Arrival:

Frozen cells must be thawed immediately upon receipt and grown according to the handling procedures described here in this instruction manual to ensure the best cell viability.

*Note: **Avoid refreezing or repetitive freezing cells upon receipt** as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures described in this instruction manual.

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:

Addexbio 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 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 the centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125xg for 5 to 7 minutes.

4. Resuspend cell pellet with the recommended complete medium and dispense into a new 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

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containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended.

Handling Procedure for Cells in Flask Culture:

The flask was seeded with cells grown and completely filled with complete medium at AddexBio facility that acts as a cushion and to prevent loss of cells during shipping.

1. Upon receipt, carefully examine if the majority of the cells are attached to the bottom of the flask using an inverted microscope (preferably equipped with phase-contrast optics), as the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable) during shipping. In addition, visually examine the culture for macroscopic evidence of any microbial contamination.
2. **For the cells are still attached**, acceptically remove all but 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **For the portion of cells that are not attached**, acceptically remove the entire contents of the flask but 10 ml of the shipping medium and centrifuge at 125 x g for 5 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to the same 25 cm^2 flask (T25). Incubate at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
4. Cells will need some time to recover from the shipping journey. Keep watching the cells and replace medium (10 ml) every two days without disturbing the monolayer for the first week or until they are 80-85% confluent.

References for C2C12 cells:

1. Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature. 1977 Dec 22-29;270(5639):725-727.

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Lot Specific Information Sheet for AddexBio Cat #: P0028001

Lot Number: 0020348

Designation: C2C12 CELLS

Total Cells/vial: >3.2×10^6

Expected Viability: 86.2-92.0%

Ampule Passage #: 15

Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)

Volume/Ampule: 1 mL

A T-25 setup at a dilution of 1:10, using culture medium as described in the product information sheet, reaches approximately 70-80% confluence within 24 hours.

Remarks:

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. Myotube formation is evident 3-4 days after switching to horse serum. FBS will take longer to achieve.

It is very important that these cells be cultured in the recommended medium or medium that contains the same formulation.

Myotube Formation:

Myotube formation can be done by allowing the cells to become confluent. A T75 flask is seeded and should be 80% to 90% confluent. The cells are then monitored and fed with fresh complete medium only as needed to keep the cells alive. After a total of 14 days incubation there should be myotube formation. The myotubes appears as think tubular structures, sometimes multinucleated.

These cells will differentiate at confluence alone, but changing to horse serum after reaching 100% confluency encourages faster and more obvious myogenesis by reducing the number of growth factors available to the cells. We are aware that myotube formation is enhanced when the medium is supplemented with horse serum instead of using FBS.

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 C2C12 cell line will become depleted rapidly if the cultures are allowed to become confluent, this can significantly delay the differentiation of these cells.

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